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Prognostic significance of Twist, ZEB1 and Slug in peripheral T-cell lymphomas

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\textbf{ABSTRACT}

\textbf{Objectives:} To investigate the protein expression of the epithelial-mesenchymal transition-inducing transcription factors (TFs) Twist, ZEB1 and Slug in peripheral T-cell lymphomas (PTCL) and their correlation with clinical parameters.

\textbf{Methods:} The expression of these TFs was studied in 53 diagnostic biopsy specimens of several different PTCL subtypes with immunohistochemistry. Patient data were retrospectively collected from patient records and a statistical analysis was performed.

\textbf{Results:} All three TFs were widely expressed. ZEB1 and Slug had correlations with clinical outcome. In all PTCL cases, high nuclear ZEB1 percentage correlated with a favorable progression-free survival (PFS) (3-year PFS: 70\% vs. 34\%; \(P = 0.010\)) and strong nuclear Slug intensity correlated with an unfavorable PFS (3-year PFS: 17\% vs. 62\%; \(P = 0.036\)).

\textbf{Discussion:} The correlations between PFS and ZEB1 or Slug protein expression have not previously been established in PTCLs. The impact of ZEB1 and Slug expression on prognosis differed from our findings in DLBCL and the impact of ZEB1 expression was in line with current studies on mycosis fungoides and Sézary syndrome. The findings may be explained by the roles these TFs play in hematopoiesis.

\textbf{Conclusion:} ZEB1 and Slug may have potential clinical value for evaluating prognosis in PTCLs. The study size was small and heterogeneous, and larger studies are warranted.

\section*{Introduction}

Peripheral T-cell lymphomas (PTCLs) are a group of rare and generally aggressive neoplasms that comprise less than 10\% of all Non-Hodgkin lymphomas in western countries. Their biology is complex with significant overlap between different entities in regard to their morphological and immunophenotypic characteristics [1,2]. The transcription factors (TFs) Twist (TWIST1), ZEB1 and Slug (SNAI2) induce epithelial-mesenchymal transition (EMT) and regulate metastasis and other pro-oncogenic functions in carcinomas, but they also play a role in lymphocyte development [3–8]. We previously studied these TFs in diffuse large B-cell lymphoma (DLBCL) and mycosis fungoides (MF) and found that Zeb1 expression correlated with an unfavorable outcome in DLBCL but with a favorable outcome in MF, whereas Twist correlated with an unfavorable outcome in MF and cytoplasmic Slug expression correlated with a favorable outcome in DLBCL [9,10]. In the present study, we used immunohistochemical staining to study the expression of Twist, ZEB1 and Slug in different subsets of PTCLs. Results were correlated with retrospectively collected relevant clinical data.

\section*{Materials and methods}

\textbf{Patients and samples}

The study population consisted of 53 patients with newly diagnosed PTCL: 22 patients had angioimmunoblastic T-cell lymphoma (AITL), 9 had peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), 7 had enteropathy associated T-cell lymphoma (EATL) and 15 had anaplastic large cell lymphoma (ALCL). The median patient age was 68 years. Diagnostic work-up included medical history and physical examination, blood chemistry, bone marrow biopsy and aspiration and whole-body computed tomography. Paraffin-embedded tissue blocks were available from all cases (53 in total). Most patients received cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) chemotherapy, or another CHOP-type regimen such as cyclophosphamide, vincristine and prednisone (COP) or CHOP plus etoposide.
Clinical data were collected retrospectively from patient records. Full clinical data were available from 44 patients and is presented in Table 1. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Immunohistochemical staining and statistical analysis

Immunohistochemistry for Twist, ZEB1 and Slug was performed as described in our previous article [9]. Nuclear and cytoplasmic staining were evaluated separately. Staining percentage of positive cells in lymphoma infiltrates and staining intensity (0–3, 0 being negative and 3 indicating strongest intensity) were evaluated separately in representative areas by an expert hematopathologist. Micrographs of immunohistochemical staining patterns were obtained using an Olympus BX41 microscope equipped with an Olympus DP11 digital camera (Olympus, Center Valley, USA). To import micrographs an HP Photo and Imaging software package (Hewlett-Packard Company, Palo Alto, USA) was used. For statistical analysis, cases were divided into four groups based on the staining results: High (>25%) and low (≤25%) percentage groups, and weak (0–1) and strong (2–3) intensity groups. Statistical analysis was performed as described previously [9].

Results

Nuclear staining

We assessed the nuclear staining of lymphoma infiltrates in 53 PTCL cases. Nuclear Twist staining was seen in 83% (44/53) of cases. Nuclear Twist percentage varied between 1% and 70%. Nuclear Twist intensity varied from 1 to 3. Nuclear ZEB1 percentage varied between 0.5% and 100%. Nuclear ZEB1 intensity varied from 1 to 3. Nuclear Slug percentage varied between 1% and 70%. Nuclear Slug intensity varied from 1 to 3.

Table 1. Patient demographics.

|                      | ALCL, ALK+, n (%) | ALCL, ALK-, n (%) | ALCL, ALK US, n (%) | EATL, n (%) | PTCL-NOS, n (%) | AITL, n (%) |
|----------------------|-------------------|-------------------|--------------------|-------------|----------------|------------|
| Number of cases      | 3                 | 8                 | 4                  | 7           | 9              | 22         |
| Female               | 1/3 (33%)         | 4/8 (50%)         | 1/4 (25%)          | 3/7 (43%)   | 4/9 (44%)      | 7/22 (32%) |
| Patients with B-symptoms | 2/3 (67%)   | 6/8 (75%)         | 0/4 (0%)           | 6/7 (86%)   | 2/9 (44%)      | 4/14 (22%) |
| Stage III–IV         | 3/3 (100%)        | 6/7 (86%)         | 0/0 (0%)           | 7/7 (100%)  | 6/9 (67%)      | 19/22 (86%)|
| Age 60 years or older| 2/3 (67%)         | 7/8 (88%)         | 3/4 (75%)          | 4/7 (57%)   | 5/9 (56%)      | 17/22 (77%)|
| Elevated LDH         | 2/3 (67%)         | 5/6 (83%)         | 1/4 (25%)          | 4/5 (80%)   | 7/9 (78%)      | 16/22 (73%)|
| WHO performance score 2 or higher | 2/3 (67%) | 4/7 (57%)         | 1/4 (25%)          | 3/7 (43%)   | 2/9 (22%)      | 9/22 (41%) |
| IPI                   |                   |                   |                    |             |                |            |
| IPI 0–1, low risk    | 0/3 (0%)          | 0/5 (0%)          | 2/4 (50%)          | 2/5 (40%)   | 2/9 (22%)      | 1/22 (5%)  |
| IPI 2–3, moderate risk| 2/3 (67%)        | 4/5 (80%)         | 1/4 (25%)          | 2/5 (40%)   | 4/9 (44%)      | 14/22 (64%)|
| IPI 4–5, high risk   | 1/3 (33%)         | 1/5 (20%)         | 1/4 (25%)          | 1/5 (20%)   | 3/9 (33%)      | 7/22 (32%) |
| Chemotherapy          |                   |                   |                    |             |                |            |
| CHOP-type             | 2/3 (67%)         | 4/8 (50%)         | 3/4 (75%)          | 4/6 (67%)   | 8/9 (89%)      | 19/22 (86%)|
| IVE                  | 0/3 (0%)          | 0/8 (0%)          | 0/4 (0%)           | 2/6 (33%)   | 0/9 (0%)       | 0/22 (0%)  |
| Other                | 1/3 (33%)         | 0/8 (0%)          | 1/4 (25%)          | 0/6 (0%)    | 1/9 (11%)      | 1/22 (5%)  |
| No chemotherapy/palliative care | 0/3 (0%)   | 4/8 (50%)         | 0/4 (0%)           | 0/6 (0%)    | 0/9 (0%)       | 2/22 (9%)  |
| Stem cell transplant  |                   |                   |                    |             |                |            |
| Autologous           | 0/3 (0%)          | 1/8 (13%)         | 1/4 (25%)          | 3/6 (50%)   | 1/8 (13%)      | 5/22 (23%) |
| Allogenic            | 1/3 (33%)         | 0/8 (0%)          | 0/4 (0%)           | 0/6 (0%)    | 0/8 (0%)       | 2/22 (9%)  |

Cytoplasmic staining

We assessed the cytoplasmic staining of lymphoma infiltrates in 53 PTCL cases. Cytoplasmic Twist staining was negative in every case except for one (staining percentage 100%, intensity 2). Cytoplasmic ZEB1 staining was negative in every case. Cytoplasmic Slug staining was positive in 86% (44/51) of cases. Cytoplasmic Slug percentage varied between 1% and 70%. Nuclear Slug intensity varied from 1 to 3. In cases with Slug staining failed and was not redone because of insufficient material.

Mutual correlations between Twist, ZEB1 and Slug

Staining intensity and staining percentage were positively correlated in all cases with discernible staining. Cytoplasmic Slug percentage positively correlated with nuclear ZEB1 intensity (P = 0.010) and nuclear Twist intensity (P = 0.044). Nuclear Slug percentage inversely correlated with nuclear ZEB1 percentage (P = 0.009).
Correlations of Twist, ZEB1 and Slug with clinicopathological parameters

An age <60 years correlated with strong nuclear Twist intensity ($P = 0.025$). Strong nuclear ZEB1 intensity correlated with the presence of B-symptoms (fever, night sweats or weight loss) ($P = 0.006$).

Correlations of Twist, ZEB1 and Slug with different PTCL subtypes

Figure 1 shows representative examples of immunohistochemical staining of PTCLs. Immunohistochemical staining results of the different PTCL subtypes are summarized in Table 2.

Prognostic value of Twist, ZEB1 and Slug

In all PTCL cases collectively, high nuclear ZEB1 percentage correlated with a favorable progression-free survival (PFS) (3-year PFS: 70% vs. 34%; $P = 0.010$). In all PTCL cases collectively, strong nuclear Slug intensity correlated with an unfavorable PFS (3-year PFS: 17% vs. 62%; $P = 0.036$) (Figure 2).

In all PTCL cases collectively, there was a nonsignificant trend toward a favorable PFS in cases with high nuclear Twist percentage ($P = 0.088$).

Discussion

In the present study, we investigated the expression of Twist, ZEB1 and Slug in PTCLs. The expression patterns delineated groups of patients with a distinctly poor prognosis. For example, the 3-year PFS was only 17% for patients with strong nuclear Slug intensity lymphomas. Physiologically these TFs induce EMT during embryological development and regulate metastasis and other pro-oncogenic functions in carcinomas [3–8, 11]. These findings regarding solid tumors are not directly applicable to lymphomas due to fundamental differences in malignant transformation and progression [12]. Correlations between the clinical course of lymphomas and the expression of Twist, ZEB1 and Slug have recently been identified and several studies have also revealed biochemical alterations related to these TFs in lymphomas [8–10,13–15].

Our previous studies of Twist, ZEB1 and Slug in DLBCL and MF found that nuclear ZEB1 expression
correlated with an unfavorable outcome in DLBCL but with a favorable outcome in MF. In the present study, ZEB1 correlated with a favorable outcome in PTCLs collectively. This was not unexpected as MF and PTCLs both originate from T-cells. ZEB1-deficient mice exhibit severe defects in T-cell differentiation, have reduced numbers of T-cells and thymocytes, and frequently develop CD4+ T-cell malignancies. Moreover, ZEB1 downregulation contributes to resistance to TGF-β-induced growth inhibition, and ZEB1 expression may inhibit the growth rate of malignant T-cells [6,13,15,16]. We found no convincing evidence of ZEB1 expression being more common in CD4+ neoplasms. For example, ZEB1 expression was often seen in PTCL-NOS which is mostly a CD4+ lymphoma. Our findings on ZEB1 were also in line with the recent genomic studies that have provided strong evidence that ZEB1 functions as a tumor suppressor in SS [17].

In contrast to ZEB1, nuclear Slug expression was associated with a poor PFS in all PTCLs collectively. This was interesting as presently there is little data concerning this TF in lymphomas. Our previous work found Slug to be associated with favorable outcome in DLBCL, but the correlation was with cytoplasmic expression and not with nuclear expression [9]. Slug protects irradiated hematopoietic progenitor cells from p53-mediated apoptosis by repressing PUMA and may also be considered an activator of hematopoietic stem cell migration, as hematopoiesis is impaired in Slug-deficient mice and migration is impaired c-kit

Table 2. Overall results of immunohistochemical staining of each PTCL subtype.

|                      | All PTCLs, n (%) | ALCL, ALK+, n (%) | ALCL, ALK-, n (%) | ALCL, ALK US, n (%) | EATL, n (%) | PTCL-NOS, n (%) | AITL, n (%) | P     |
|----------------------|-----------------|------------------|------------------|-------------------|------------|----------------|------------|-------|
| Nuclear Twist staining |                 |                  |                  |                   |            |                |            |       |
| High percentage      | 16/53 (30%)     | 0/3 (0%)         | 2/8 (25%)        | 2/4 (50%)         | 1/7 (14%)  | 5/9 (56%)      | 6/22 (27%) | NS    |
| Strong intensity     | 18/53 (34%)     | 2/3 (67%)        | 2/8 (25%)        | 3/4 (75%)         | 1/7 (14%)  | 4/9 (44%)      | 5/22 (23%) | NS    |
| Nuclear ZEB1 staining |                 |                  |                  |                   |            |                |            |       |
| High percentage      | 31/53 (58%)*    | 1/3 (33%)        | 5/8 (63%)        | 2/4 (50%)         | 3/7 (43%)  | 8/9 (89%)      | 12/22 (55%) | NS    |
| Strong intensity     | 23/53 (43%)     | 1/3 (33%)        | 3/8 (38%)        | 4/4 (100%)        | 5/7 (71%)  | 6/9 (67%)      | 4/22 (18%) | 0.009 |
| Nuclear Slug staining |                 |                  |                  |                   |            |                |            |       |
| High percentage      | 4/51 (8%)       | 1/3 (33%)        | 0/7 (0%)         | 2/4 (50%)         | 0/7 (0%)   | 0/9 (0%)       | 1/21 (5%)  | 0.011 |
| Strong intensity     | 11/51 (22%)†    | 2/3 (67%)        | 1/7 (14%)        | 3/4 (75%)         | 0/7 (0%)   | 3/9 (33%)      | 2/21 (10%) | 0.010 |
| Cytoplasmic Slug staining |               |                  |                  |                   |            |                |            |       |
| High percentage      | 42/51 (82%)     | 1/3 (33%)        | 7/7 (100%)       | 4/4 (100%)        | 7/7 (100%) | 9/9 (100%)     | 14/21 (67%) | NS    |
| Strong intensity     | 21/51 (41%)     | 1/3 (33%)        | 3/7 (43%)        | 1/4 (25%)         | 4/7 (57%)  | 6/9 (67%)      | 6/21 (29%) | NS    |

NS indicates not statistically significant; US indicates unspecified.

*Significant correlation with a better PFS.
†Significant correlation with a poorer PFS.

Figure 2. Kaplan-Meier curves showing differences in PFS. (A–C) PFS in PTCLs collectively based on (A) percent ZEB1+ nuclei within tumors (P = 0.010; number of cases: 44) and (B) nuclear Slug intensity (P = 0.036; number of cases: 43).
expressing cells [4,7,18]. The correlation with adverse outcomes we observed could be partly explained by the antiapoptotic functions of Slug. On the other hand, such correlation was not seen in DLBCL [9]. Presently, Slug has not been extensively studied in lymphomas and we believe that further investigations are warranted.

Physiologically, Twist is widely expressed in CD34+ stem cells and generally has an antiapoptotic function in malignancies; it antagonizes the p53 pathway and prevents c-myc-induced apoptosis, also repressing the NF-kB pathway. In T-cell lymphomas, Twist expression is increased in advanced MF/SS lesions [8,19–21] and correlates with an unfavorable prognosis in MF. We did not find statistically significant correlations between Twist expression and disease outcome in PTCLs.

In summary, we found that immunohistochemical detection of the EMT-related ZEB1 and Slug can be used to delineate a subset of PTCLs with a distinctly poor prognosis. Our study had several limitations and needs to be validated with a larger patient population. The sample size was small, and we were not able to establish significant correlations within the different disease groups. The heterogeneity of PTCLs must also be mentioned. For example, EATL has different risk factors and clinical course when compared toAITL. Some of the cases did not have full data available, and in four older ALCL cases, we were unable to reliably establish the ALK-status. Recently published research on Twist, ZEB1 and Slug in T-cell lymphoma has focused on CTCLs, but their impact on PTCLs has not been yet established. Our findings describe the expression of these TFs in PTCLs and provide a novel insight on the possible prognostic value of studying ZEB1 and Slug expression.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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References
[1] Vose JM, Neumann M, Harris ME. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes international T-cell lymphoma project. J Clin Oncol. 2008;26:4124–4130. DOI:10.1200/JCO.2008.16.4558
[2] Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:2375–2391. DOI:10.1182/blood-2016-01-643569
[3] Montserrat N, Gallardo A, Escuin D, et al. Repression of E-cadherin by SNAIL, ZEB1, and TWIST in invasive ductal carcinomas of the breast: a cooperative effort? Hum Pathol. 2011;42:103–110. DOI:10.1016/j.humpath.2010.05.019
[4] Haupt S, Alsheich-Bartok O, Haupt Y. Clues from worms: a Slug at puma promotes the survival of blood progenitors. Cell Death Differ. 2006;13:913–915. DOI:10.1038/sj.scd.4401906
[5] Zhang P, Sun Y, Ma L. ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. Cell Cycle. 2015;14:481–487. DOI:10.1080/15384101.2015.1006048
[6] Nakahata S, Yamazaki S, Nakauchi H, et al. Downregulation of ZEB1 and overexpression of Smad7 contribute to resistance to TGF-B1-mediated growth suppression in adult T-cell leukemia/lymphoma. Oncogene. 2010;29:4157–4169. DOI:10.1038/onc.2010.172
[7] Wu WS, Heinrichs S, Xu D, et al. Slug antagonizes p33-mediated apoptosis of hematopoietic progenitors by repressing puma. Cell. 2005;123:641–653. DOI:10.1016/j.cell.2005.09.029
[8] Goswami M, Duvic M, Dougherty A, et al. Increased Twist expression in advanced stage of mycosis fungoides and Sézary syndrome. J Cutan Pathol. 2012;39:500–507. DOI:10.1111/j.1600-0560.2012.01883.x
[9] Lemma S, Karihtala P, Haapasaa KM, et al. Biological roles and prognostic values of the epithelial-mesenchymal transition-mediating transcription factors Twist, ZEB1 and Slug in diffuse large B-cell lymphoma. Histopathology. 2013;62:326–333. DOI:10.1111/his.12000
[10] Häyrinen MJ, Uotila PM, Sahi H, et al. Twist and Zeb1 expression identify mycosis fungoides patients with low risk of disease progression. J Eur Acad Dermatol Venereol. 2019. DOI:10.1111/jdv.16009
[11] Dong CY, Liu XY, et al. Twist-1, a novel regulator of epithelial-mesenchymal transition, metastasis and therapy resistance. J cell. 2005.09.029
[12] Chen SC, Liao TT, Yang MH. Emerging roles of epithelial-mesenchymal transition-mediating transcription factors Twist, ZEB1 and Slug in hematopoietic malignancies. J Biomed Sci. 2018;25(37). DOI:10.1186/s12929-018-4221
[13] Hidaka T, Nakahata S, Hatakeyama K, et al. Down-regulation of TCF8 is involved in the leukemogenesis of adult T-cell leukemia lymphoma. Blood. 2008;112:383–393. DOI:10.1182/blood-2008-01-131185
[14] Sánchez-Tilló E, Fanlo L, Siles L, et al. The EMT activator ZEB1 promotes tumor growth and determines differential response to chemotherapy in mantle cell lymphoma. Cell Death Differ. 2014;21:247–257. DOI:10.1038/cdd.2013.123
[15] Higashiy M, Moribe H, Takagi T, et al. Impairment of T-cell development in deltaEF1 mutant mice. J Exp Med. 1997;185:1467–1479. DOI:10.1084/jem.185.8.1467
[16] Caprini E, Bresin A, Cristoforetti C, et al. Loss of the candidate tumor suppressor ZEB1 (TCF8, ZFHX1A) in Sézary syndrome.
[17] Pérez-Losada J, Sánchez-Martín M, Rodríguez-García A, et al. Zinc-finger transcription factor Slug contributes to the function of the stem cell factor c-kit signaling pathway. Blood. 2002;100:1274–1286.

[18] Merindol N, Riquet A, Szablewski V, et al. The emerging role of twist proteins in hematopoietic cells and hematological malignancies. Blood Cancer J. 2014;4:e206–e212. DOI: 10.1038/BCJ.2014.22

[19] Maestro R, Dei Tos AP, Hamamori Y, et al. Twist is a potential oncogene that inhibits apoptosis. Genes Dev. 1999;13:2207–2217. DOI: 10.1101/gad.13.17.2207

[20] Koh HS, Lee C, Lee KS, et al. Twist2 regulates CD7 expression and galectin-1-induced apoptosis in mature T-cells. Mol Cells. 2009;28:553–558. DOI: 10.1007/s10059-009-0150-8

[21] Šošić D, Richardson JA, Yu K, et al. Twist regulates cytokine gene expression through a negative feedback loop that represses NF-kappaB activity. Cell. 2003;112:169–180. DOI: 10.1016/S0092-8674(03)00002-3