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Cyclin D1 Expression in Peripheral T-cell Lymphomas

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Abstract

Cyclin D1 is an important regulator of the cell cycle and overexpression of this protein by immunohistochemistry is characteristically seen in mantle cell lymphoma as well as other B-cell neoplasms. However, little is known about the expression of this protein in T-cell lymphomas. Cyclin-dependent kinase pathway inhibitors are in development, therefore identifying cyclin D1-positive T-cell lymphomas may provide a therapeutic target in a disease where novel treatments are urgently needed. We collected 200 peripheral T-cell lymphomas from three institutions including the following types of cases: 34 anaplastic large cell lymphoma, ALK+, 44 anaplastic large cell lymphoma, ALK negative, 68 peripheral T-cell lymphomas, not otherwise specified, 24 angioimmunoblastic T-cell lymphomas, 7 extranodal NK/T-cell lymphomas, 4 enteropathy associated T-cell lymphomas, 3 hepatosplenic T-cell lymphomas, 12 cutaneous T-cell lymphomas, and 4 large granular lymphocytic leukemias. Immunohistochemical stains for cyclin D1 protein (SP4 clone) were performed on paraffin-embedded tissue. In a subset of cases, *IGH/CCND1* fluorescence *in situ* hybridization analysis was also performed. Cyclin D1 staining was predominantly seen in anaplastic large cell lymphoma, including 8 of 34 cases with ALK+ anaplastic large cell lymphoma (24%), and 3 of 44 cases of ALK negative (7%) anaplastic large cell lymphoma. Three cases of peripheral T-cell lymphoma, not otherwise specified, were also positive (3/68, 4%). All other T-cell lymphomas were negative for cyclin D1. In four of the cyclin D1-positive T-cell lymphomas by immunohistochemistry, fluorescence *in situ* hybridization analysis was negative for *IGH/CCND1* translocation or extra copies of the *CCND1* gene. Cyclin D1 overexpression by immunohistochemistry is not limited to B-cell lymphomas and is also observed in some peripheral T-cell lymphomas, particularly in anaplastic large cell lymphoma, ALK+. Cyclin D1 expression was not associated with extra copies or translocation of the *CCND1* gene. Cyclin D1 over-expression may be the result of a post-translational phenomenon and may

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represent a potential therapeutic target using agents that target the cyclin-dependent kinase pathway.

INTRODUCTION

The cyclin proteins are important in the regulation of the cell cycle by activating cyclin-dependent kinases at specific stages of the cell cycle, and thus promoting DNA replication and cell division. The cyclin D class of proteins are closely related to the G1 cyclins and activate cyclin-dependent kinase 4 and cyclin-dependent kinase 6 (1). Mitogenic stimuli activate the tyrosine kinase receptor which triggers a signaling cascade, resulting in upregulation of cyclin D1 (2, 3). The deregulation of cyclin-dependent kinases can lead to cancer by causing proliferation that is independent of the checkpoints and regulators (1, 4). Overexpression of cyclin D1 protein is implicated in many tumors such as mantle cell lymphoma, non-small cell lung cancer, plasma cell myeloma, hairy cell leukemia, as well as breast and esophageal cancers(1). Genomic alterations such as the t(11;14)(q13;q32), which juxtaposes the cyclin D1-encoding gene (*CCND1*) to the immunoglobulin heavy chain gene are characteristic for mantle cell lymphoma (5) but is also seen in plasma cell myeloma(6).

Although cyclin D1 protein expression is well characterized in solid tumors and B-cell lymphomas, there are little data regarding cyclin D1 expression in peripheral T-cell lymphomas (7, 8). In this retrospective study we evaluated the prevalence of cyclin D1 expression in peripheral T-cell lymphomas. The identification of cyclin D1 has treatment implications because cyclin D1 can be a therapeutic target.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded tissue from patients with a diagnosis of peripheral T-cell lymphoma was identified from the archives of three institutions (City of Hope National Medical Center, Mayo Clinic, and the University of Chicago). Institutional Review Board approval was obtained for this study at each center.

Four micron thick sections of formalin-fixed, paraffin-embedded tissue were cut from whole sections as well as tissue microarray blocks that were subsequently deparaffinized in xylene and hydrated using serial percentages of alcohol. Antibodies directed against cyclin D1 (clone SP4; 1:20 dilution, Thermo Fisher Scientific, Fremont, CA, USA), anti-cyclin D2 (clone aa1–30; 1:250 dilution, LSBio, Seattle, WA, USA), cyclin D3 (clone DCS22; 1:200 dilution, Cell Signaling Technology, Danvers, MA, USA), phospho-Stat3 (Tyr705) (M9C6, 1:50 dilution, Cell Signaling Technology, Danvers, MA, USA), and SOX11 (clone MRQ-58; 1:100 dilution, Leica, Buffalo Grove, IL, USA) were used on these tissue sections. All stains were performed on the BOND immunostainer by Leica (Buffalo Grove, IL, USA).

The above immunohistochemical stains were optimized on formalin-fixed, paraffin-embedded tissue with normal tonsils as the control. For all stains, a cutoff of ≥20% was used to assign as positive. Three pathologists (JYS, LS, and AF) independently scored all cases and any discrepant cases were resolved by re-scoring on a multi-headed microscope.

Fluorescence *in situ* hybridization was performed on formalin-fixed, paraffin-embedded tissue sections from whole tissue blocks and tissue microarrays using a previously reported method (9). Hybridization with the *CCND1/IGH* dual color, dual fusion probe (Abbott Molecular, Des Plaines, IL) was used. Two hundred interphase nuclei were analyzed for each specimen analyzed. Two *CCND1/IGH* fusion signals (yellow) were scored as positive for *CCND1/IGH* gene rearrangement.

RESULTS

We identified 200 peripheral T-cell lymphomas from the three institutions and classified them using the World Health Organization Classification(10). The cases included: 34 anaplastic large cell lymphoma, ALK-positive, 44 anaplastic large cell lymphoma, ALK-negative, 68 peripheral T-cell lymphomas, not otherwise specified, 24 angioimmunoblastic T-cell lymphomas, 7 extranodal NK/T-cell lymphomas, 4 enteropathy-associated T-cell lymphomas, 3 hepatosplenic T-cell lymphomas, 12 cutaneous T-cell lymphomas, and 4 large granular lymphocytic leukemias.

The clinical data on these cases were limited because many were referred only for hematopathology consultation or because clinical follow up information was not available. Only two patients that were cyclin D1-positive had clinical follow up and both had anaplastic large cell lymphoma, ALK+. One patient is in remission two years after initial treatment and the other patient went into remission after chemotherapy and autologous stem cell transplantation but relapsed two years later and subsequently underwent allogeneic stem cell transplantation, had relapsed disease, and was then treated with vorinostat and is in complete remission.

Cyclin D1 staining was predominantly seen in anaplastic large cell lymphoma, ALK+ (8/34, 24%) and anaplastic large cell lymphoma, ALK negative (3/44, 7%, p=0.05) (Tables 1). Four of the anaplastic large cell lymphoma cases had diffuse nuclear staining (3 anaplastic large cell lymphoma, ALK+, 1 anaplastic large cell lymphoma, ALK-negative) (Figure 1), while 7 cases (5 anaplastic large cell lymphoma, ALK+, 2 anaplastic large cell lymphoma, ALK-negative) showed scattered nuclei positive but greater than 20% of the nuclei. The background endothelial cell nuclei were also positive and attention was taken not to confuse them with the neoplastic cells. Three of 68 cases (4%) of peripheral T-cell lymphoma, not otherwise specified, were also positive (Figure 2); CD30 was negative in two of these cases and showed partial weak staining in one. All other peripheral T-cell lymphomas were negative for cyclin D1 protein expression. In the three diffusely cyclin D1-positive anaplastic large cell lymphoma, ALK+ cases and one peripheral T-cell lymphoma, not otherwise specified case by immunohistochemistry, fluorescence *in situ* hybridization analysis was subsequently performed and was negative for *IGH/CCND1* translocation as well as extra copies of the *CCND1* gene (Figure 2E). SOX11 staining was also negative on all cyclin D1-positive cases by immunohistochemistry.

Due to the higher incidence of cyclin D1 protein expression in anaplastic large cell lymphoma, additional immunohistochemical stains were performed on this group that had available additional material to investigate the JAK/STAT pathway. The majority of

anaplastic large cell lymphoma, ALK+ cases were positive for cyclin D2 (10/12, 83%) and cyclin D3 (12/12, 100%). As for anaplastic large cell lymphoma, ALK-negative cases, cyclin D2 (7/15, 47%, p=0.28), and cyclin D3 (5/15, 33%, p=0.03) were less frequently positive. As expected, phospho-STAT3 (pSTAT3) was expressed in more cases of anaplastic large cell lymphoma, ALK+ (13/13, 100%) compared to ALK-negative cases (6/16, 38%, p=0.039) (Figures 3 and 4).

DISCUSSION

Cyclin D1 protein expression is commonly seen in B-cell neoplasms, classically in mantle cell lymphoma but also in hairy cell leukemia and plasma cell myeloma. In this study, we show that the expression of cyclin D1 is not limited to B-cell lymphomas but can also be seen in some peripheral T-cell lymphomas, particularly anaplastic large cell lymphoma and is not associated with translocation or extra copies of the *CCND1* gene.

In our study, cyclin D1 protein expression was highest in the anaplastic large cell lymphoma, ALK+ cases (24%) compared to ALK-negative (7%) and peripheral T-cell lymphoma, not otherwise specified (4%). Normally, the cell cycle is tightly regulated by molecules such as the cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors. Cyclin D1 overexpression induces G1 phase progression by activating cyclin-dependent kinases which phosphorylates substrates such as RB and other transcription factors that are important in proliferation and differentiation (3, 4, 11–13). In anaplastic large cell lymphoma, ALK+, the *NPM-ALK* fusion protein binds to and phosphorylates STAT3, which results in constitutive activation (14–16). STAT3 targets many genes including the anti-apoptotic proteins (e.g. Bcl-xL, Bcl-2), as well as proliferation-associated proteins such as myc and cyclin D1 (17, 18). Constitutively-activated STAT3 has been shown to promote tumorigenesis and has been found to increase cyclin D1 and myc mRNA levels approximately 3–5 fold in transformed cells(18).

Phospho-STAT3 was more commonly expressed in anaplastic large cell lymphoma, ALK + (100%) as compared to ALK-negative (38%, p=0.039). Interestingly, in the anaplastic large cell lymphoma cases that were ALK+ and expressed pSTAT3, most of these cases expressed two or more of the cyclin D proteins (83%), whereas only two (13%) of the ALK-negative and pSTAT3-positive cases expressed any cyclin D proteins. Cyclin D3 expression in anaplastic large cell lymphomas has been described before and our findings are consistent with the notion that activation of STAT3 in ALK-positive cases leads to the overexpression of the cyclin proteins promoting cell proliferation(19).

Prior studies investigating cyclin D1 expression in peripheral T-cell lymphoma reported negative findings, likely due to the limited number of cases evaluated (e.g. only 3–7 cases of anaplastic large cell lymphoma, ALK+)(20, 21). Only a few cases of anaplastic large cell lymphoma, ALK+, showed diffuse nuclear staining for cyclin D1 protein, whereas other cases showed only focal staining in the neoplastic cells. Many of the negative cases showed positivity in the background endothelial cells. Unfortunately, clinical follow up on the majority of our cyclin D1-positive T-cell lymphomas was unavailable and therefore, it is unclear whether cyclin D1 protein expression impacts prognosis.

Although cyclin D1 overexpression is not specific to any particular cancer, it is important in oncogenesis and proliferation of many tumors such as squamous cell carcinomas of the head and neck(22), breast cancers(23), and colorectal carcinomas(24). Cyclin D1 has been frequently associated with some specific subtypes of B-cell lymphomas but, as our study shows, can also been in peripheral T-cell lymphomas. The overexpression of cyclin D proteins is seen preferentially in anaplastic large cell lymphoma, ALK+, most likely due to activation of the JAK/STAT pathway(25). Routine staining for cyclin D1 protein in peripheral T-cell lymphomas will identify patients who may benefit from therapies that target cyclin D1 or its associated cyclin-dependent kinases(13, 26).

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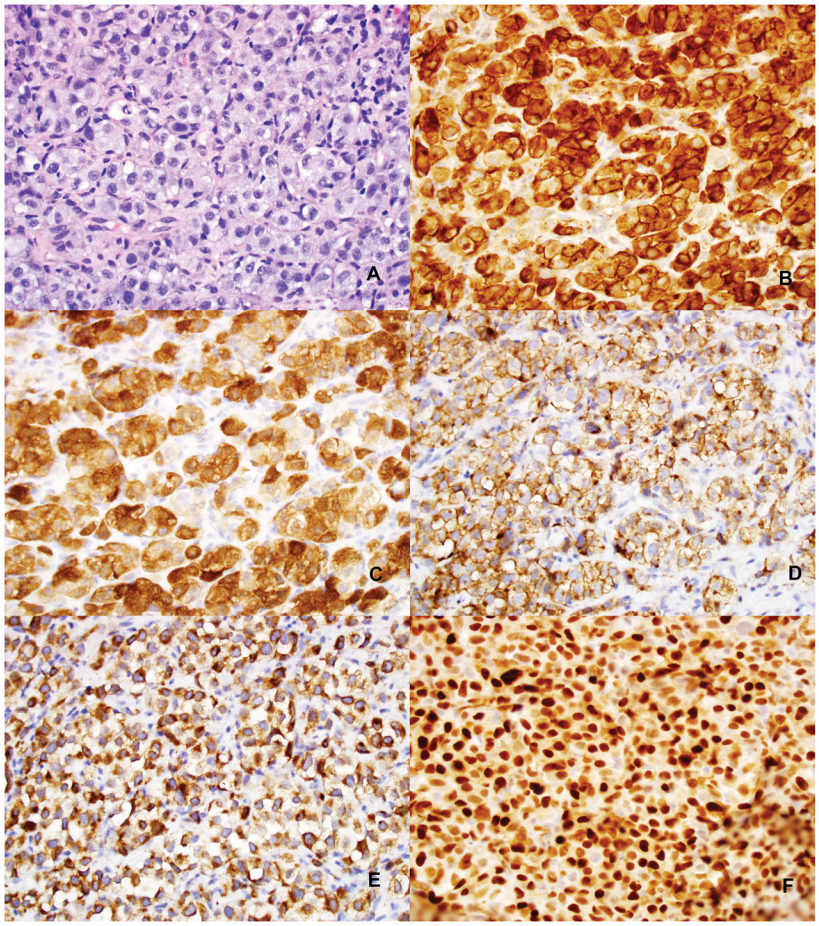

Figure 1.

Anaplastic large cell lymphoma, ALK+. **A)** H&E stain showing pleomorphic cells in sheets. **B)** These cells are strongly positive for CD30, **C)** ALK protein (nuclear and cytoplasmic), **D)** CD4, **E)** perforin, and **F)** cyclin D1 by immunohistochemistry.

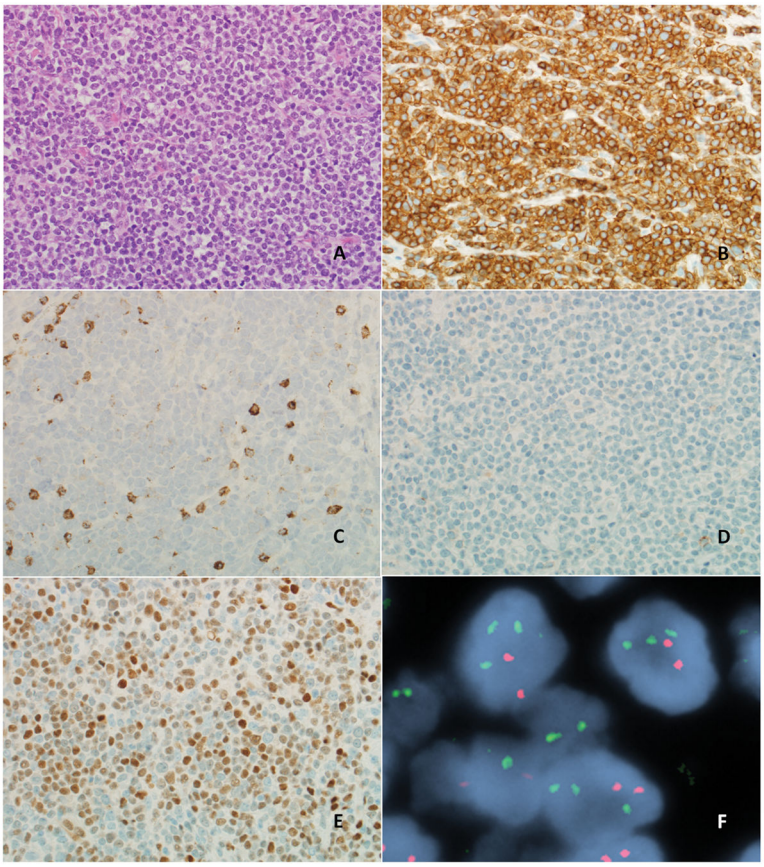

Figure 2.

Peripheral T-cell lymphoma, not otherwise specified. **A)** Sheets of mostly small tumor cells (H&E). **B)** The tumor cells are positive for CD3, and **C)** show aberrant loss of CD2. **D)** CD30 is negative. **E)** Cyclin D1 is positive in many of the tumor cells. **F)** Fluorescence *in situ* hybridization using a dual-fusion probe for *CCND1* (red) and *IGH* (green) shows no evidence of *IGH-CCND1* fusion or other abnormality of *CCND1*. There is an extra copy of the *IGH* locus. Fluorescence *in situ* hybridization image courtesy of Reid Meyer, Mayo Clinic, Rochester, MN.

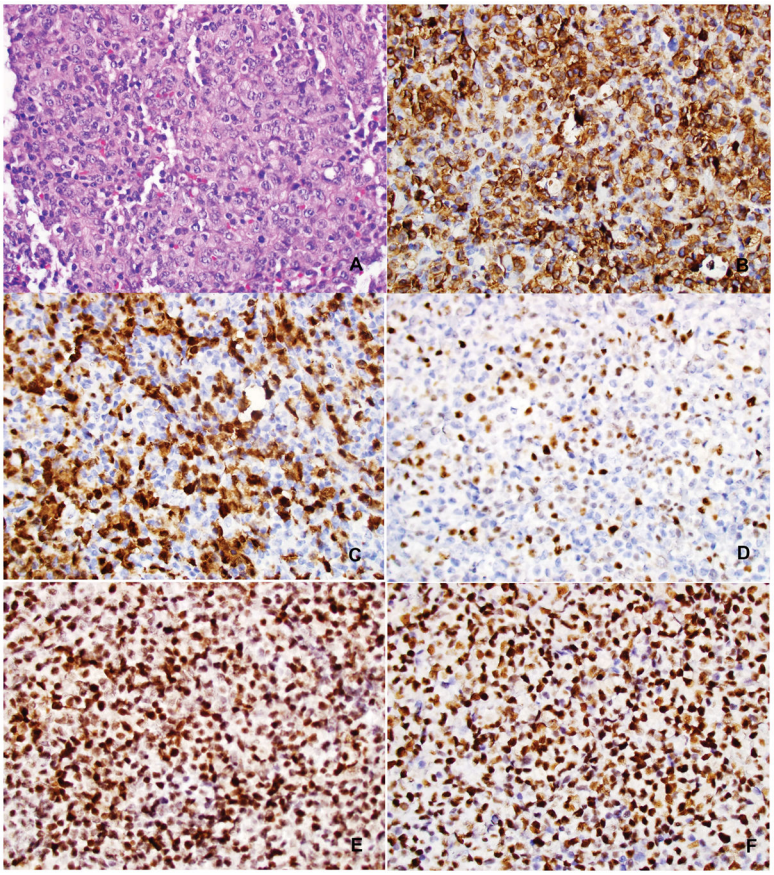

Figure 3.

Anaplastic large cell lymphoma, ALK+. **A)** Sheets of tumor cells with pleomorphic morphology (H&E). **B)** These cells are positive for CD30, **C)** ALK1 protein, **D)** focal cyclin D1, **E)** cyclin D2, and **F)** phospho-STAT3. The cells were also diffusely positive nuclear staining cyclin D3 (not shown).

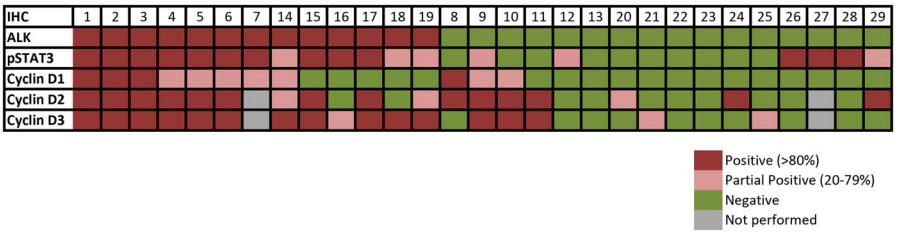

Figure 4.

Immunohistochemical staining pattern of anaplastic large cell lymphoma

IHC: Immunohistochemistry

Table 1

Peripheral T-cell lymphomas immunostaining pattern

T-cell lymphoma subtype	No. (%)			
	Cyclin D1	Cyclin D2	Cyclin D3	pStat3
Anaplastic large cell lymphoma, ALK+	8/34 (24%)	10/12 (83%)	12/12 (100%)	13/13 (100%)
Anaplastic large cell lymphoma, ALK-	3/44 (7%)	7/15 (47%)	5/15 (33%)	6/16 (38%)
Peripheral T-cell lymphoma, NOS	3/68 (4%)			
Angioimmunoblastic T-cell lymphoma	0/24 (0%)			
Extranodal NK/T-cell lymphoma, nasal type	0/7 (0%)			
Enteropathy associated T-cell lymphoma	0/4 (0%)			
Hepatosplenic T-cell lymphoma	0/3 (0%)			
Cutaneous anaplastic large cell lymphoma	0/5 (0%)			
Mycosis fungoides	0/5 (0%)			
Subcutaneous panniculitis-like T-cell lymphoma	0/1 (0%)			
CD4-positive pleomorphic T-cell lymphoma	0/1 (0%)			
T-cell large granular lymphocytic leukemia	0/4 (0%)			
Overall	**14/200 (7%)**			

* p value between ALCL, ALK+ and ALCL, ALK-negative for cyclin D1 (p=0.05), cyclin D2 (p=0.28), cyclin D3 (p=0.03), and pSTAT3 (p=0.039)