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The Role of AHI1 and CDKN1C in Cutaneous T-Cell Lymphoma Progression

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Abstract

Cutaneous T-cell lymphoma (CTCL) is the most common lymphoma of the skin. Recent reports suggest that AHI1 is overexpressed in a subset of CTCL-derived cell lines, where it downregulates the expression of CDKN1C tumor suppressor gene. In the current work, we test the expression of these genes in 60 patients with Mycosis Fungoides and Sezary Syndrome by RT-PCR and correlate our findings with 6 years of clinical follow-up. These findings reveal that AHI1 and CDKN1C exhibit opposite expression patterns, where AHI1 is expressed in poor and intermediate prognosis patients, while CDKN1C is expressed in favourable prognosis patients. Kaplan–Meier analysis documents that downregulation of CDKN1C is associated with poor disease outcome in patients with CTCL, while upregulation of AHI1 shows a weak association with aggressive disease course.

Keywords

Cutaneous T Cell Lymphoma (CTCL); gene expression; AHI1 and *CDKN1C*

Background

Cutaneous T-cell lymphoma (CTCL) represents a heterogeneous group of non-Hodgkin lymphomas with Mycosis Fungoides (MF), Cutaneous Anaplastic Large Cell Lymphoma (C-ALCL) and Sezary Syndrome (SS) being some of the most common forms [1]. The molecular pathogenesis of CTCL remains unknown. Early identification of patients at risk of progression would allow for earlier use of more aggressive therapies. Unfortunately, currently there are no validated molecular/biological markers available to predict which patients with early stage CTCL will progress.

However, emerging research is starting to elucidate such prognostic markers [2]. Notably, it was suggested that PCR analysis of T-cell receptor rearrangements in lesional CTCL skin

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may correlate with poor disease prognosis if the same structural rearrangements are detected in multiple skin biopsies at the time of diagnosis [3]. Additional studies using biomarkers are also underway to establish how CTCL treatments impact lesional skin [4]. More importantly, recent experimental studies revealed that Abelson Helper Integration site 1 (AHI1) and downstream signaling members, including Cyclin-Dependent Kinase Inhibitor 1C (CDKN1C), may play an important role in CTCL carcinogenesis [5]. AHI1 is commonly activated by provirus insertional mutagenesis in various murine leukaemias and lymphomas and was found to be significantly upregulated in Hut102 and Hut78 CTCL cell lines [5, 6]. Experimental work demonstrated that one of the functions of AHI1 in Hut78 cells is to suppress several genes including the tumor suppressor gene, CDKN1C. To date, it remains unknown whether AHI1 and/or CDKN1C are expressed in lesional skin in patients with CTCL and whether their expression is important for CTCL disease progression.

In the past, we performed microarray and RT-PCR analyses of gene expression in biopsy specimens from 60 stage I–IV MF and SS patients[7, 8]. These patients were prospectively followed for 6 years. The initial RT-PCR analysis revealed three distinct transcription profile clusters (i.e. clusters 1, 2 and 3), where clusters 1 and 3 contained a mix of stage I–IV disease patients, while cluster 2 contained mostly stage I and only a few cases of advanced disease patients. All stage IV MF and SS patients fell into clusters 1 and 3 [7]. The described three distinct transcription profile clusters were associated with different clinical courses. Cluster 2 genes associated with the best clinical outcome and good response to therapy while cluster 1 and 3 genes associated with the worst and intermediate clinical outcomes, respectively, and poor response to therapy [7, 8]. More importantly, this analysis revealed a number of prognostic molecular markers that correlated with poor (e.g. IL-17F) vs. favorable (e.g. WIF1) disease course in early stage CTCL patients [7]. Unfortunately, the initial analysis did not include AHI1 and CDKN1C genes.

Questions addressed

In the current work, we tested the expression of AHI1 and CDKN1C genes in lesional skin from 60 patients with CTCL and correlated such gene expression with disease progression.

Experimental design

Patients and samples

All patients were enrolled in an IRB-approved study with informed consent. Chart review was conducted for all patients to collect information on clinical parameters and outcomes between January 2003 and January 2009. Disease progression was defined as progression to the next clinical stage and/or death. For this study, the revised 2005 ISCL/EORTC disease classification was used [9]. All tissue samples were obtained and processed as previously described [8]. The biopsy samples analyzed in this report are the same samples that were analyzed by microarray and RT-PCR in the previous studies [7, 8].

Quantitative RT-PCR gene expression analysis

RT-PCR was performed as previously described [7]. Primers for AHI1 and CDKN1C genes were designed using Primer 3 software [10] and were purchased from Invitrogen (AHI1

forward primer 5′GTCCAAAACTACCCCATCAAGGCT3′ and reverse primer 5′GCAGCACAGGAACGTATCACCT3′; CDKN1C forward primer 5′AGATCAGCGCCTGAGAAGTCGT3′ and reverse primer 5′CTCGGGGCTCTTTGGGCTCT3′). RT-PCR was performed utilizing the obtained cDNA from patients with CTCL and iScript RT-PCR mix (Bio-Rad, Mississauga, Ontario, Canada) on Bio-Rad iCycler as previously described [7]. The expression was standardized using geNorm method utilizing ACTB, SDHA and B2M housekeeping genes [11]. The obtained data were analyzed using XLSTAT software to obtain Kaplan–Meier curves [12]. p values were calculated using the log-rank test [13].

Results

Patients with CTCL were enrolled in the study and followed as previously described [7]. As expected, most patients (70% or 42 of 60) were diagnosed with stage I disease at the time of biopsy. Follow-up of early stage MF patients revealed that 7 of 42 (or 16.7%) patients progressed towards more advanced stages (i.e. beyond stage I) during the 6 years in the study. On the other hand, 83% (15 of 18) of patients with advanced (stage 2B and beyond) disease progressed towards higher clinical stages and/or death.

RT-PCR findings of AHI1 and CDKN1C expression in the biopsied lesional skin showed cluster restricted expression of these genes, where AHI1 appears to be expressed in clusters 1 and 3 patients (poor and intermediate prognosis clusters), while the expression of CDKN1C appears to be primarily restricted to cluster 2 patients (favorable prognosis patients) (Fig. 1). Our previous work documented that the above-described 3-cluster signature expression pattern independently correlates with poor vs. favorable disease outcomes [7].

Consistent with these findings, Kaplan–Meier analyses show that downregulation of CDKN1C expression leads to more aggressive disease course in stages I–IV patients (Fig. 2a, $P = 0.041$). Furthermore, loss of expression of this gene in stage I patients also correlates with aggressive disease (Fig. 2b, $P = 0.023$). Consistent with the suspected effect of AHI1 on CDKN1C, we observed a trend, where high expression of AHI1 correlates with more recalcitrant disease in stages I–IV (Fig. 2c) and stage I (Fig. 2d) disease patients. Unfortunately, the Kaplan–Meier findings of AHI1 did not reach statistical significance ($P = 0.172$ and $P = 0.189$ respectively). The lack of statistical significance may be due to low number of patients, insufficient follow-up time or due to the effect of additional signaling pathways that lead to decrease in CDKN1C expression.

Conclusion

Our findings provide clinical support for a possible molecular link between the function of a putative oncogene (AHI1) and a tumor suppressor gene (CDKN1C) and suggest that the expression of CDKN1C may be important to maintain an indolent MF disease state. Further understanding of AHI1 and CDKN1C signaling may lead to the development of novel therapies for patients with MF.

Acknowledgments

Dr. Sasseville and Dr. Kupper designed the research. Dr. Litvinov performed the molecular analysis of gene expression. Dr. Sasseville, Dr. Litvinov and Dr. Kupper analyzed the data and prepared the manuscript.

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References

1. Lamberg SI, Bunn PA Jr. Arch Dermatol. 1979; 115:1103–1105. [PubMed: 39515]
2. van Kester MS, Borg MK, Zoutman WH, et al. J Invest Dermatol. 2012; 132:2050–2059. [PubMed: 22513784]
3. Vega F, Luthra R, Medeiros LJ, et al. Blood. 2002; 100:3369–3373. [PubMed: 12384439]
4. Knol AC, Quereux G, Brocard A, et al. Exp Dermatol. 2010; 19:e299–e301. [PubMed: 19845753]
5. Kennah E, Ringrose A, Zhou LL, et al. Blood. 2009; 113:4646–4655. [PubMed: 19211505]
6. Ringrose A, Zhou Y, Pang E, et al. Leukemia. 2006; 20:1593–1601. [PubMed: 16838023]
7. Litvinov IV, Jones DA, Sasseville D, et al. Clin Cancer Res. 2010; 16:2106–2114. [PubMed: 20233883]
8. Shin J, Monti S, Aires DJ, et al. Blood. 2007; 110:3015–3027. [PubMed: 17638852]
9. Slater DN. Br J Dermatol. 2005; 153:874–880. [PubMed: 16225594]
10. Rozen S, Skaletsky H. Methods Mol Biol. 2000; 132:365–386. [PubMed: 10547847]
11. Vandesompele J, De Preter K, Pattyn F, et al. Genome Biol. 2002; 3:0034.
12. Kaplan EL, Meier P. J Am Stat Assoc. 1958; 53:457–481.
13. Mantel N. Cancer Chemother Rep. 1966; 50:163–170. [PubMed: 5910392]

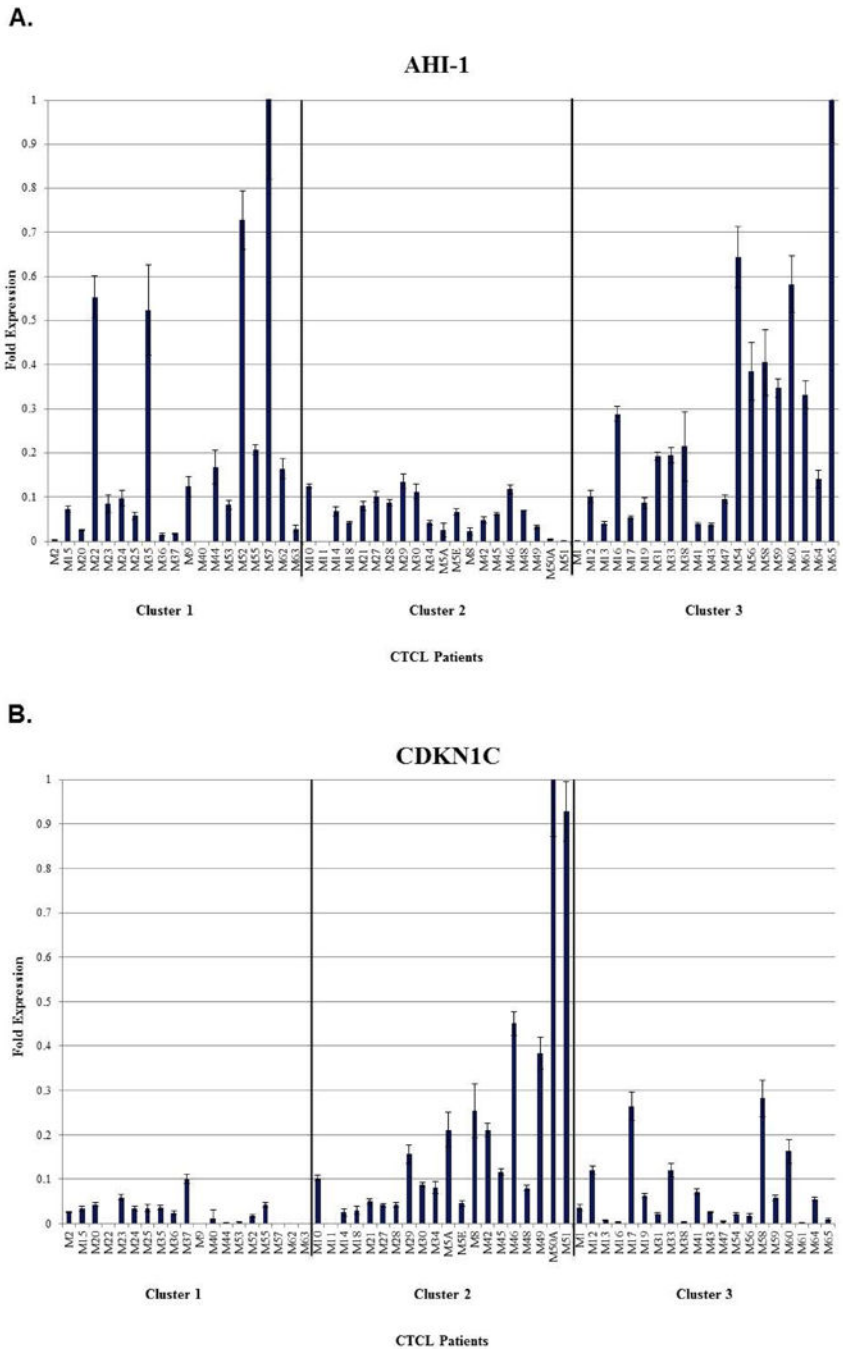

Figure 1.
Expresssion of AHI1 (A) and CDKN1C (B) in clusters 1–3 CTCL patients.

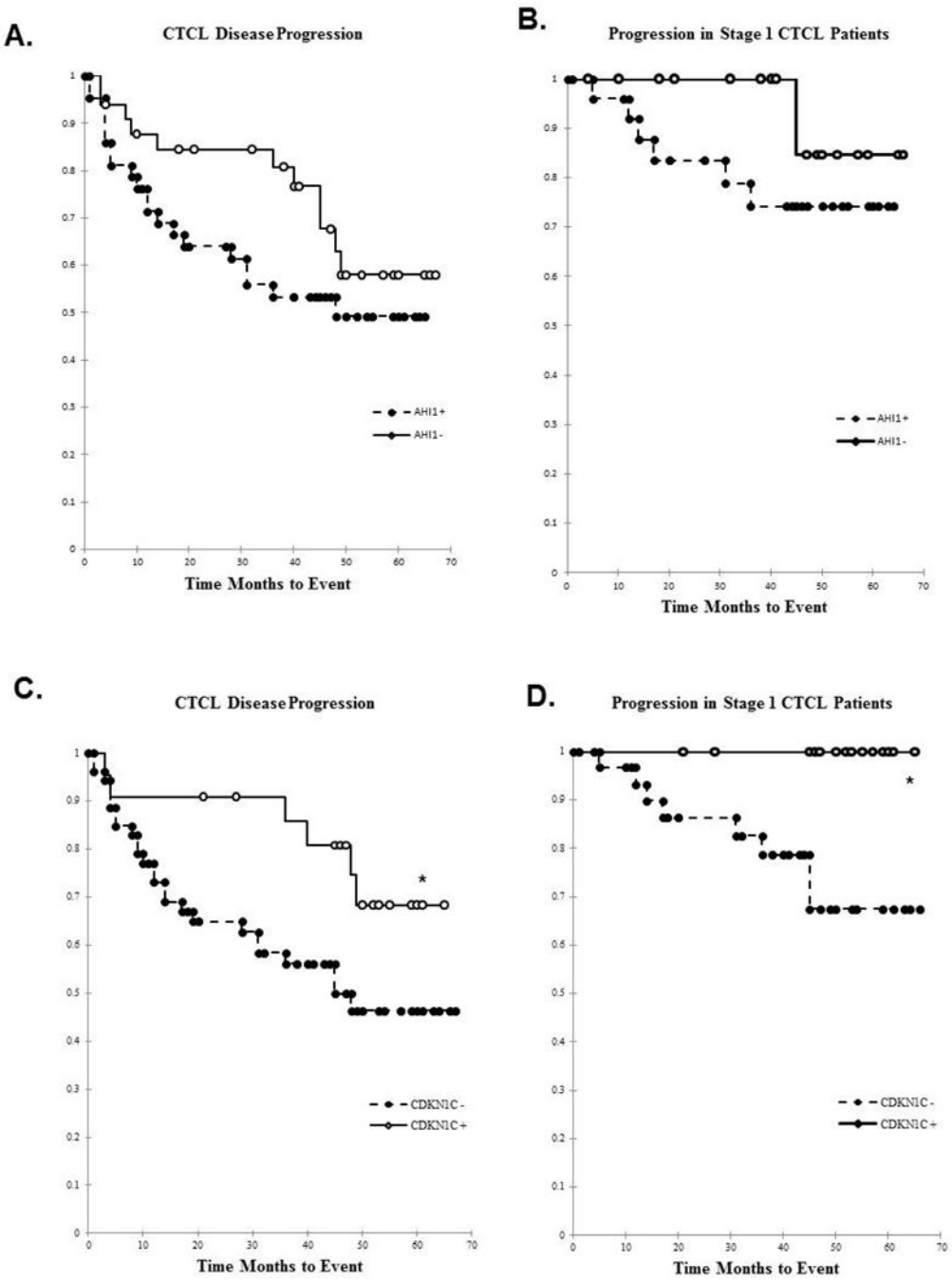

Figure 2. Correlation of disease progression with CDKN1C and AHI1 expression in patients

(a) Correlation of CDKN1C expression with the disease progression in 60 stage I–IV patients (P = 0.041). (b) Correlation of CDKN1C expression with the disease progression in 42 stage I MF patients (P = 0.023). (c) Correlation of AHI1 expression with the disease progression in 60 stage I–IV patients (P = 0.162). (d) Correlation of AHI1 expression with the disease progression in 42 stage I MF patients (P = 0.189).