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Polycomb response element-binding sites in the MDR of CLL: Potential tumor suppressor regulation

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ABSTRACT

Chronic lymphocytic leukemia [CLL] is the most common adult leukemia and is heterogeneous in clinical presentation. CLL cases present with various chromosomal aberrations, including 11q23, 14q32, 17p, and trisomy 12, with the most common abnormality being deletion of 13q14 [1]. Although monoallelic deletion of 13q14 is common, there is a subset of patients who have complete nullisomy at 13q14, a locus that has been hypothesized to contribute to CLL pathogenesis [2] due to loss of tumor suppressors [DLEU and miR-15a/16-1]. We hypothesized that deletion of both copies of 13q14 would lead to uncontrollable proliferation of CLL cells and a poor prognosis. We examined our 13q14 nullisomy for survival, treatment-free survival, lymphocyte doubling time, and the presence of lymphadenopathy. Furthermore, we compared the gene expression profiles between patients with 13q14 monosomy, nullisomy, or normal karyotype. Our results suggest that patients with 13q nullisomy have a higher incidence of bulky lymphadenopathy [16.6% compared to 10% of monosomy patients], a higher frequency of lymphocyte doubling time [27.7% compared to 7.4% of monosomy patients], and a higher rate of needing treatment [50% compared to 18.5% of monosomy patients]. We observed deletion of DLEU1 and HTR2A, consistent with a gene dosage effect, and observed PRE-binding sites on DLEU1. Patients with homozygous deletion of 13q14 had a worse prognosis compared to heterozygotes. Lastly, the DLEU1 locus is a possible “second hit” loss for CLL progression.

Keywords: Chronic Lymphocytic Leukemia; Gene Expression; 13q14; Nullisomy; DLEU; Tolerogenic

1. INTRODUCTION

Chronic lymphocytic leukemia [CLL] is the most common adult leukemia and is heterogeneous in clinical presentation. Previous studies have focused on identifying prognostic markers such as CD38− and ZAP70-expression, immunoglobulin mutational status, lymphocyte doubling time, presence of lymphadenopathy, and chromosomal abnormalities to better group patients for therapy [1,2]. Patients with CLL may have none, one, or several of the following chromosomal abnormalities: del [11q23], del [13q14], del [17p], trisomy 12, and, less frequently, del [14q32]. However, there exists a cohort of patients with loss of both alleles at 13q14 [13q null] that is distinct of patients with monosomy at this region [13q mono] and wild type [WT]. The 13q14 locus was hypothesized to contribute to CLL pathogenesis due to the loss of tumor suppressors, DLEU and miR-15a/16-1 [3-6]. We hypothesized that deletion of both copies of 13q14 would lead to uncontrollable proliferation of CLL cells and a poor prognosis. To test this hypothesis, we performed retrospective analyses with the data we accumulated from CLL patients cared for at the University of Nebraska Medical Center [UNMC].

2. PATIENTS AND METHODS

2.1. Patient Samples

All peripheral blood CLL [PB-CLL] samples were obtained by venipuncture, and the CLL cells from bone marrow [BM-CLL] and lymph node [LN-CLL] were collected from the excess tissue samples. All protocols were approved by the Institutional Review Board [IRB] and Scientific Review Committee [SRC] at UNMC. PB-CLL and BM-CLL cells were separated by using magnetic bead cell separation [Milenyi Biotec, Auburn, CA, USA], and samples with 95% or greater CD5+CD19+ cells were used for analyses. To obtain LN-CLL cells,
serial sections of frozen lymph node biopsies were obtained from the UNMC tissue bank. Immunohistochemistry was used to stain each section for CD5 and CD19 to identify areas of >90% CLL cells. The CLL cells were microdissected out for the analyses.

2.2. Fluorescent in Situ Hybridization [FISH] and Microarray Analyses

Fluorescent in situ hybridization [FISH] was used to determine chromosomal abnormalities in CLL cells as previously described [7]. The annotated patient data was examined to determine the association between time to treatment, treatment-free survival, lymphocyte doubling time, and the presence of lymphadenopathy. Microarray analyses were used to determine differential expression of genes in a region of 13q suggested to be deleted in CLL [8,9].

Previously, a tolerogenic signature was associated with poor prognosis in CLL [9-12]. Genes from the tolerogenic signature were examined among patients with 13q null, 13q mono, and WT cases. Next, the genes within the 13q locus that were included in our array were clustered by CLL patients WT at this locus compared to 13q mono and 13q null. These results would describe whether there was a gene copy effect between CLL cases with losses at this region, which would identify a candidate tumor suppressor gene[s].

2.3. Examination of Regulatory Elements

The sequence for DLEU1 was examined using Vista [pipeline.lbl.gov/cgi-bin/gateway2] and the University of California, Santa Cruz [UCSC] Genome Bioinformatics [genome.ucsc.edu] Programs. The entire coding and non-coding sequence of DLEU1 was blasted for the polycomb response elements [PRE], including: GAF, G10, PHO, and Z binding sites.

3. RESULTS AND DISCUSSION

3.1. Prognosis for CLL Patients with 13q Nullisomy

Our cohort of 13q null patients [n = 34] had divergent clinical characteristics from that of 13q mono CLL cases [n = 27]. 13q null patients had higher incidences of bulky lymphadenopathy [16.6% null compared to 0% mono], shorter lymphocyte doubling time [27.7% null compared to 7.4% mono], and greater occurrences of needing treatment [50% null, 18.5% mono; Table 1]. CLL patients were broadly defined as having a poor prognosis if there was lymph node-involvement, a short lymphocyte doubling time [defined as less than a year for doubling], and if they required treatment. Although CLL patients with 13q deletion are generally classified as having a favorable outcome, we suggest a divergence in prognosis between patients with a heterozygous, compared to a homozygous, deletion at this locus. Accordingly, patients with homozygous deletion of 13q14 might benefit from earlier treatment.

We next examined the percentage of CLL cells with 13q null to determine their susceptibility to treatment. The FISH results from pre-treatment and post-treatment for CLL patients showed a decrease in the number of CLL cells positive for 13q14 nullisomy from 18% pre-treatment to only 5% patients after treatment (p = 0.015; Figure 1(a)). This 73% decline preliminarily suggests that early treatment of patients with 13q null could be beneficial.

3.2. Gene Expression Profiles [GEP] of Patients with 13q Nullisomy

In order to determine whether there was a divergence in GEP between WT, 13q null, and 13q mono cases, microarray data were examined. Previously, we reported that a tolerogenic signature is indicative of patients with an unfavorable outcome compared to those with a more indolent disease [8]. Furthermore, based on clinical prognostic indicators, our results suggested that CLL patients with 13q14 null had a less favorable prognosis compared to those mono or WT. As a consequence, we examined the tolerogenic GEP between CLL cases WT, mono, or null at 13q14 to determine if this expression signature correlated with clinical outcome. We hypothesized that patients with 13q14 null would have higher expression of the tolerogenic signature compared to 13q mono or WT cases.

Changes in the GEP were compared between 13 null, 13q mono, and patients WT at the 13q14 locus (Figure 1(b)). There was an increase in the immunosuppressive...
3.3. Gene Expression in CLL Cases Nullisomy, Monosomy, or WT at 13q14

After determining a prognostic significance for complete loss of 13q14, the next objective was to identify whether a tolerogenic/immunosuppressive signature was differentially expressed in these cells. Supervised cluster analyses were performed on the genes included in our microarray platform that were within the 13q14 locus [8; MWG Biotech, Germany, Human 10K oligo set A; Figure 1(c)]. The expected result was to observe a dosage effect of genes within the deleted region at 13q14. To determine critical genes in the region of interest, we expected patients WT at the locus to have high expression [red], those missing one copy [+/−] to have a lower expression [lighter red or green], and individuals null at this region to have no expression of critical genes [black]. We anticipated the results of these analyses would determine a region that was commonly deleted in CLL that we could compare to other reports in the literature.

The expression of two genes was consistent with the predicted expression pattern; these were HTR2A and DLEU1 ([Figure 1(c)]. The location of HTR2A and DLEU1 is depicted in Figure 1(d). 5-hydroxytryptamine [serotonin] receptor 2A [HTR2A], G-protein-coupled receptor, has never been reported as having a role in CLL. Furthermore, as serotonin functions as a neurotransmitter, its association with cancer pathophysiology is an interesting observation. The gene deleted in lymphocytic
leukemia 1 [DLEU1] is a long non-coding RNA [lncRNA] previously reported to participate in CLL [21-25]. Although there are only 15 published papers describing DLEU1 to date, early reports and observations show promise that the regulation of this lncRNA appears to have a great influence on CLL progression.

Although the role of DLEU1 in CLL is still elusive, we searched the sequence of this region to gain greater insight. A polycomb response element [PRE] conserved sequence was identified, known to serve as an antagonist for epigenetic regulation of gene expression [26] within this region (Figure 2(a)). This region is a regulatory switchable element that influences the architecture of chromatin and the expression of nearby genes [27,28]. In this regard, DLEU1 is potentially a docking site for the polyhomeotic [PHO] protein. This evolutionarily conserved, regulatory system was identified in Drosophila. The mammalian homolog of PHO in Drosophila is the

![Figure 2. Polycomb response element binding site: (a) Putative PRE binding sites on the long noncoding RNA, DLEU1 at 13q14. The binding sites for the GAGA factor (GAF; 29), G10, extended GAGA site (G10; 30), PHO consensus (31), and Zeste binding site (Z; 30) are highlighted in the selected portion of the sequence as indicated by the legend. Due to the length of DLEU1, an abbreviated portion of the sequence is provided in this figure; (b) Hypothesized mechanism of regulation by DLEU1. DLEU1, a long non-coding RNA (lncRNA) deleted in CLL with unknown function. We have identified PRE binding sites on DLEU1 and describe a hypothesized mechanism of DLEU1 regulating transcription to maintain homeostasis, but when DLEU1 is lost as in the case of 13q null, then transcription is dysregulated and multiple “hits” to the
transcription factor YY1 [29]. Recently, a lncRNA was the
link between copy number variation and a polycomb/ 
thriothorax epigenetic switch in muscular dystrophy [26], 
and we suspect a similar switch may be occurring in 
CLL following the loss of regulation of the region con-
taining DLEU1 (Figure 2(b)). This finding suggests a 
potential negative feedback loop with the binding of YY1 
to DLEU1 thereby regulating gene expression. Therefore, 
this finding would explain the over decade-long conun-
drum of observing a deletion in this region, but being 
unable to identify a clear point mutation or tumor sup-
pressor gene.

In summary, this report describes an unfavorable 
prognosis for patients with biallelic deletion of 13q14, 
compared to 13q mono patients. However, the per-
centage of CLL cells with 13q null decreased after treatment, 
suggesting that chemotherapy was effective at killing 
these malignant cells. GEP of patients with 13q null, 
compared to 13q mono, identified the overexpression 
APC and TGFβ1. Therefore, the upregulation of these 
immunoregulatory molecules in 13q null patients might 
lead to a greater immunosuppression in these cases.

The limitation of this study is the number of cases 
examined. A larger cohort is needed to conclusively 
determine the biological relevance of DLEU1 in CLL cases 
with 13q14 null, but hopefully this study will serve as a 
basis for additional in-depth analyses. Previously, a 
similar study with a large sample size [n = 323] showed 
that a higher percentage of 13q null cells were associated 
with a significantly shorter time to treatment [6]. Simi-
larly, we suggest that CLL patients with 13q null have a 
worser prognosis than patients that are mono or WT at 
13q14. Contrasting these, data contradict those de-
scribed earlier this year [30]. Garg et al. observed that 
the baseline characteristics between CLL cases with mono- 
or bi-allelic deletion of 13q differed only by 
ZAP70-expression and albumin levels [31]. This study 
differs from ours in that solely fluorescent in situ 
hybridization [FISH] was used to assess the genetic abnor-
malities in CLL cells. Patients included in our study 
were first screened using both FISH and gene expression 
profiling. Therefore, this study presents a comprehensive 
picture as to the genetic abnormalities present within this 
frequently deleted region.

It will be difficult to prove a role for the consensus 
sequence of DLEU1 in CLL without engineering a dele-
tion at that region both in CLL samples and an in vivo 
animal model. While this manuscript was in preparation, 
Lia et al. (2012) published a report regarding this precise 
question [31]. Transgenic mice engineered to have loss 
of 13q14 had a shorter life expectancy, similar to our 
data from patients described in this report [31: Table 1]. 
The data presented herein suggest that, first, CLL cases 
show a heterozygous deletion at 13q14, and then a “sec-
ond hit” or loss of the second allele [13q null] is neces-
sary to take CLL from an indolent to an aggressive stage. 
Prospective studies are necessary to conclusively deter-
mine this switch causing CLL cells to drive a more ag-
gressive disease.

4. SUMMARY

The findings presented herein are significant in that they 
suggest a different way of classifying CLL cases with 
13q null. However, it is important to note the limitations 
with the sample size described in this study. Based on 
this small sample size, we suggest that patients with the 
deletion of both copies of 13q14 potentially need therapy 
initiated earlier than their heterozygous counterparts and, 
thus, might require more frequent monitoring of lymph-
ocyte counts. The influence of DLEU1 in CLL was 
reported previously, and the microRNAs in this region 
have been studied extensively [2-5]. However, this is the 
first report suggesting that regulatory elements, specifi-
cally PRE-sequences, of DLEU1 might contribute as a 
“second hit” to lead to a more aggressive disease pro-
gression for patients with CLL.

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REFERENCES

[1] Mraz, M., Pospisilova, S., Malinova, K., Slapak, I. and 
Mayer, J. (2009) MicroRNAs in chronic lymphocytic leu-
kenia pathogenesis and disease subtypes. Leukemia & 
Lymphoma, 50, 506-509. 
doi:10.1080/10428190902763517

vivo lentiviral delivery of miR-15a/16 reduces malignant-
cy in the NZB de novo mouse model of chronic lympho-
doi:10.1038/gene.2011.58

frequently deleted in malignancy, functions as a critical 
host gene of the cell cycle inhibitory microRNAs miR-
15a and miR-16-1. Experimental Cell Research, 315, 
2941-2952. doi:10.1016/j.yexcr.2009.07.001

mir-15a/miR-16 cluster controls B cell proliferation and its 
deletion leads to chronic lymphocytic leukemia. Cancer 
Cell, 17, 28-40. doi:10.1016/j.ccr.2009.11.019

13q14 deletions in CLL involve cooperating tumor sup-
doi:10.1182/blood-2009-10-249367


