Cytogenetic, molecular, and array-based analysis of a complex translocation found in a patient diagnosed with CLL

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Cytogenetic, molecular, and array-based analysis of a complex translocation found in a patient diagnosed with CLL

Submitted in partial fulfillment of the requirements for graduation with honors to the Department of Natural Sciences at Carroll College, Helena, Montana

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April 12, 2011
This thesis for honors recognition has been approved for the Department of Natural Sciences by:

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Acknowledgments

I would like to thank Dr. Thomas Dennis for his guidance and support through this research project, as well as for developing the ideas and strategies involved. I thank Amy Zearfoss for her expert help in cytogenetic analysis and her good humor. I thank Sandy Phillips for her assistance in FISH analysis and Katie Styren for her help in aCGH operation and analysis. I would also like to thank Glynis Scott for her assistance in cell culturing and preparation. Additionally, I would like to thank Shodair Children’s Hospital for the research opportunity and tools necessary to complete it. I would like to thank Dr. John Addis for his overall immense support and Dr. Colin Thomas for his constructive comments. I would like to express gratitude to my sister, Riley Gallogly, for her inescapable encouragement. Finally, I would like to thank my family for their unending moral support throughout this research endeavor.
# Table of Contents

Acknowledgements...........................................................................................................iii

Abstract............................................................................................................................................................v

List of Figures...................................................................................................................................................vi

Introduction.......................................................................................................................................................1

Materials and Methods.................................................................................................................................3

Results..............................................................................................................................................................5

Discussion.........................................................................................................................................................21

Literature Cited..............................................................................................................................................25
Abstract

Although a number of genes and chromosomal abnormalities have been associated with the presence of chronic lymphocytic leukemia (CLL), no oncogenes or critical tumor suppressor genes (TSGs) have yet been implicated in its onset. In this study, we describe a chromosomal abnormality existing in a subset of white blood cells from an individual experiencing early stages of CLL: a balanced rearrangement involving the p-arms of chromosomes 1, 3, and 6. This mutation being both unique and balanced suggests that the genes disrupted by the translocation breakpoints may be critical to the initiation of CLL. Microdissection techniques were used to physically isolate chromosomal breakpoint regions, which were then analyzed via array comparative genomic hybridization and results confirmed by fluorescence in-situ hybridization. The translocation breakpoints were narrowed to contain WAS protein family member 2 (WASF2), AT hook DNA binding motif (AHDC1), and Gardner-Rasheed feline sarcoma viral oncogene homolog (FGR) genes on chromosome 1, a TSG cluster on chromosome 3, and the UHRF1 binding protein 1 (UHRF1BP1) gene on chromosome 6. Earlier research found that the FGR gene and 3p21.3 TSG cluster have properties that may promote neoplastic tendencies during abnormal expression. In conclusion, we suggest one or more of these genes is a candidate for initial and/or early development of CLL. Future work will be focused on further narrowing the breakpoints of this rearrangement and studying cellular implications involved in abnormal expression of these candidate genes.
List of Figures

Figure 1. Metaphase spread and karyotype of a white blood cell with a chromosome 1, 3, 6 translocation...............................................................8

Figure 2. Metaphase spread before and after microdissection.................................9

Figure 3. FISH images showing PCR product scraped via microdissection of chromosome 1..............................................................................10

Figure 4. FISH images showing PCR product scraped via microdissection of chromosome 3..............................................................................11

Figure 5. Ideograms of normal and rearranged chromosomes 1, 3, and 6..............12

Figure 6. Microarray data from sample 1 across entire chromosome 1.................13

Figure 7. Microarray data from sample 1, magnified image at breakpoint on chromosome 1..............................................................................14

Figure 8. Microarray data from sample 2 across entire chromosome 1...............15

Figure 9. Microarray data from sample 2, magnified image at breakpoint on chromosome 1..............................................................................16

Figure 10. Microarray data from sample 2 across entire chromosome 3.............17

Figure 11. Microarray data from sample 2, magnified image at breakpoint on chromosome 3..............................................................................18

Figure 12. Microarray data from sample 1 across entire chromosome 6.............19

Figure 13. Microarray data from sample 1, magnified image at breakpoint on chromosome 6..............................................................................20
Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the world, currently affecting approximately 100,760 people in the United States [1]. The disease causes a slow overproduction of B cells in the bone marrow and blood, which in turn crowd out healthy white blood cells and red blood cells. The initiating factors of CLL and primary oncogenes involved are currently unknown [2]. Cytogenetic analyses of CLL affected B-cell chromosomes often show deletions in the long arm of 13, deletions in the short and long arms of chromosome 17, and trisomy 12 [3]. Although one or more of these cytogenetic mutations may indicate CLL, it is generally accepted that none of these defects are the initiating cause of CLL, but are instead secondary mutations [3,4].

Analyzing the breakpoints of chromosomal rearrangements is often used to identify direct consequences of translocations, insertions, and deletions. Breakpoints of rearrangements may split functional genes or disrupt areas of gene expression, causing improper gene function. Many oncogenes and other cancer related genes have been discovered through precise mapping of chromosomal rearrangements [5]. Several common leukemias are known to be caused by disruption of oncogenes via balanced translocations, notably acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). Types M2 and M3 AML are caused by a t(8;21) translocation involving RUNX1/RUNX1T1 genes and a t(15;17) translocation involving RARA/PML genes, respectively [6,7]. CML is initiated by the Philadelphia translocation, t(9;22), which disrupts the BCR/ABL genes. On rare occasions, individuals diagnosed with CLL may have novel genetic mutations normally not observed [4]. If these mutations are balanced rearrangements and exist with no other secondary genetic mutations, the observed rearrangement may be the primary cause of the cellular neoplastic development. Identification of the genes disrupted by the rearrangement may prove invaluable to the understanding of CLL and the cellular progression of the disease.

Array comparative genomic hybridization (aCGH) is a powerful tool used to quickly and accurately analyze human DNA for genetic mutations and variations. This type of microarray analysis uses tens of thousands of oligonucleotide probes to simultaneously detect regions across the entire human genome. Laser analysis of the probes reveals single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) [8]. Because balanced translocations do not have gains or losses in genetic material, microarray analysis does not detect the mutation. This problem can be circumvented using microdissection techniques. With use of a microscopic capillary needle and a micromanipulator, entire chromosomes or specific regions on chromosomes can be physically scraped from a metaphase spread on a glass microscope slide. The removed DNA can then be amplified using PCR techniques and analyzed using aCGH. It has been shown that microarray CNV analysis will detect losses throughout the entire genome and gains in regions that were microdissected, as only the microdissected regions are analyzed and are compared against a normal control genome.
The breakpoints of a balanced translocation can therefore easily be determined with high accuracy when the region of interest is microdissected. In this study, a novel balanced translocation found in an individual diagnosed with CLL was analyzed using cytogenetic microdissection and aCGH in order to identify genes located at the breakpoints and look for evidence of oncogenic functionality.
Materials and Methods

Sample

The sample was obtained as left-over material following clinical cytogenetic analysis. The patient diagnosis, sample type, and white blood cell (WBC) count were obtained and used in the study. The identity of the patient from which the sample was obtained was completely removed from the sample and data upon initiation of the study and cannot be recovered. The sample used in this study was a bone marrow extraction and had a WBC concentration greater than 100,000 cells/ml. Cells were harvested, cultured, and prepared as metaphases on glass microscope slides using standard procedures provided by the genetics lab of Shodair Children’s Clinic (Helena, MT).

Microdissection

Microdissection of chromosomal target regions was done using a Zeiss (Oberkochen, Germany) microscope modified with a hydraulic joystick for controlling movement of a capillary needle. Microdissection needles were prepared from borosilicate glass capillary tubes using a model 720 Kopf needle/pipette puller (Tujunga, CA). Needles were UV irradiated, removing contaminating DNA/RNA. Target regions on chromosomes were identified by means of computer enhanced metaphase karyotype analysis, and photographed before and after microdissection. Target regions of DNA were scraped with a capillary needle and transferred to collection solution. Eight to twelve microdissection scrapes were performed on separate metaphases for the region of interest.

PCR Amplification

Microdissected products were initially PCR amplified using a low temperature DNA polymerase, rather than Taq polymerase, and topoisomerase in order to increase chances of primer annealing. During the first eight rounds of PCR, Sequenase™ Version 2.0 DNA polymerase (USB, Cat. No. 70775Y, Cleveland, OH) was added manually to each round during primer annealing. Degenerative oligonucleotide primers (Midland Certified Reagent Co, Midland, Texas) were used in PCR, containing the sequence 5’-CCGACTCGAGNNNNNATGTGG-3’. PCR began at 37°C for 30 minutes, allowing Topoisomerase I (Promega, Cat. No. M2851, Madison, WI) to unwind target DNA. PCR temperature was raised to 93°C for 4 min for initial denaturation. Eight cycles of PCR followed using a 1 minute denaturation step at 94°C, a 2.5 minute annealing step at 30°C, and a 2.5 minute extension step at 37°C.

The PCR product from the first eight rounds then underwent a second PCR reaction, using AmpliTaq LD® DNA polymerase (Applied Biosystems, Cat. No. N808-0107m, Carlsbad, CA). PCR was initiated with a 4 minute denaturation step at 93°C. Thirty PCR cycles followed using a 1 minute denaturation step at 94°C, a 1 minute annealing step at 56°C, and
a 2 minute extension step at 72°C. After the final round of PCR, the temperature was held at 72°C for 5 minutes to complete extension. Temperature was then lowered to 4°C indefinitely.

Portions of the amplified DNA from the second PCR reaction were labeled with Digoxigenin-11-dUTP (Roche, Cat. No. 11093088910, Mannheim, Germany) for FISH confirmation and Biotin-16-dUTP (Roche, Cat. No. 11093070910, Mannheim, Germany) for microarray preparation. The Digoxigenin-11-dUTP and Biotin-16-dUTP labels were incorporated into the DNA using PCR; concentrations of thymidine present in the PCR reaction solution were decreased by 50%, thus allowing the uracil based labels to have less competition and easier DNA incorporation during extension. The PCR cycles for labeling are identical to the second PCR reaction described earlier.

PCR products were cleaned using 30 kDa Microcon centrifugal filters (Sigma-Aldrich, Cat. No. Z648068, St. Louis, MO) and ethanol precipitation. Following 24 hours of ethanol precipitation, ethanol was completely removed using vacuum centrifugation and DNA was rehydrated with sterile water.

**Fluorescence in situ Hybridization (FISH) Confirmation**

Blood slides were prepared for FISH probe hybridization using de-identified blood pellets that were clinically determined to contain no mutations and were otherwise normal. The slides were unstained and were not exposed to immersion oil or ethidium bromide. Hybridization solution was made using Digoxigenin-11 labeled DNA, Hybrisol VII (MP Biomedical, Cat. No. RIST1390, Solon, OH), and Human Hybloc DNA (AGL, Cat. No. HHB, Melbourne, FL). Hybridization solution was pipetted on prepared FISH slides, covered with a coverslip, and placed on a ThermoBrite StatSpin Hybrite (Abbott Molecular, Abbott Park, IL) for 16 hours. The slides were then post-washed and labeled with anti-Dig antibody (Roche, Cat. No. 11207741910) and Prolong Gold DAPI (Invitrogen, Cat. No. P36935, Carlsbad, CA). Slides were analyzed using a spectrum green filter on a Zeiss Axio Imager 2 microscope. Slides were stored in a dark -20°C freezer.

**Microarray**

Biotin labeled DNA was hybridized to microarray chips using standard Affymetrix (Santa Clara, CA) microarray procedures. The microarray chips were post-washed, thus removing leftover DNA that did not bind to oligonucleotides on the microarray chips, then scanned on an Affymetrix Cytogenetics Whole-Genome 2.7M Array, also using standard protocols. The results were interpreted and displayed using the Chromosome Analysis Suite (Affymetrix). Genomic locations were retrieved from NCBI build 37.2.
Results

The CLL bone marrow sample obtained for this study had a WBC count greater than 100,000 cells per milliliter, indicating a large buildup of WBCs in the bone marrow. Initial cytogenetic analysis performed by clinical cyto-geneticists at Shodair Children’s Hospital showed an obvious translocation involving the p-arms of chromosomes one and three. This aberration was only visible in a fraction of metaphase spreads, suggesting it occurred in only a subset of WBCs, this being consistent with the pathology of CLL affecting exclusively B cells. The end of the p-arm of chromosome 1 was composed of an uncharacteristic single dark band, or possibly two or more bands very closely spaced (Figure 1). Visual analysis estimated the breakpoint of the translocation at 1p36. Chromosome 3 had an unusual light banding pattern on the upper region of its p-arm, correlating to the pattern normally seen on the p-arm of chromosome 1 (Figure 1). This further suggested that the translocation was balanced between chromosomes 1 and 3. The breakpoint for chromosome 3 was visually estimated at 3p21. These were the only cytogenetically visible mutations found in blood cells of the patient. As the patient was already clinically diagnosed with CLL via flow cytometry and clinical patient analysis, it was determined that this novel translocation and the genes affected by it may have directly caused the onset of CLL.

In an attempt to isolate chromosomal regions containing the breakpoints, two microdissections were performed on the chromosomes of WBC metaphases: the region ranging from the telomere of chromosome 1’s p-arm towards the centromere, visibly beyond the translocation breakpoint (referred to as sample 1); the region ranging from the telomere of chromosome 3’s p-arm towards the centromere, also visibly beyond the translocation breakpoint (referred to as sample 2). Figure 2 shows a metaphase before and after microdissection.

Following microdissection and PCR amplification, a portion of the PCR product was labeled for FISH and analyzed in order to verify success of PCR and confirm any results found by microarray analysis (aCGH). Initial FISH analysis revealed the consistent hybridization of the p-arms of four chromosomes from each PCR sample (samples 1 and 2). Using inverted DAPI (a pseudo-color applied to DAPI fluorescence designed to express G-band characteristics) and relative measures of chromosome length, it was clear that in each sample probes hybridized homologous chromosomes (Figures 3 and 4), indicating that segments from only two different types of chromosomes existed in the PCR samples. This was the expected result, as one microdissection sample should only yield product pertaining to DNA found on each side of the translocation. This verified that the PCR ran successfully and without contamination. The resolution provided by the inverted DAPI was insufficient to give definitive identification as to the natures of the chromosomes that were hybridized, but enough similarities were observed to assume that chromosomes 1 and 3 were involved.

Microarray analysis was performed on data obtained by the Affymetrix Cytogenetics Whole-Genome 2.7M Array using the Affymetrix Chromosome Analysis Suite. The breakpoints of each chromosome were determined by measuring differences in the log2
ratio of copy number variants (CNVs) and analysis of average CNVs that were detectable in each microdissection sample. The log2 ratio was determined automatically by the Chromosome Analysis Suite, and represents a two-fold change in copy number for every unit increase or decrease. Microarray analysis of CNVs on sample 1 (microdissected product of chromosome 1 p-arm) revealed no-gains in the p-arm of chromosome 1, ranging from the telomere to 1p36.1. After this point, there was a dramatic increase in CNVs, as was expected for the aCGH analysis of the translocated area. Interestingly, there were no gains in CNVs on chromosome 3 for sample 1, indicating that the region translocated to chromosome 1 was not from chromosome 3, as was initially expected. Instead, detectable gains were found in chromosome 6, ranging from the telomere to a sharp drop-off in CNVs at 6p21. Sample 2 (microdissected product of chromosome 3 p-arm) aCGH analysis indicated gains in the p-arm of chromosome 1, ranging from the telomere to 1p36.1, where there is a low resolution drop in CNVs. There were detectable losses in the p-arm of chromosome 3, from the telomere until 3p21.3, where there are significant and rapid gains in CNVs. This initial analysis by aCGH showed that, contrary to early expectations, the translocation actually involved the p-arms of chromosomes 1, 3, and 6, rather than just chromosomes 1 and 3. The three-way translocation replaced the top segment of chromosome 1 with the top segment of chromosome 6. Similarly, the translocated p-arm of chromosome 1 went to chromosome 3, and the top of chromosome 3 replaced the top of chromosome 6. An ideogram visualizes this translocation in Figure 5. Reanalysis of cytogenetic banding images and FISH images confirmed the complex translocation existing between the p-arms of chromosomes 1, 3, and 6 (Figures 1, 3, and 4). This find is particularly significant because three genes may be directly affected by this mutation. No other significant gains or losses were detected by aCGH analysis.

The actual locations of the chromosomal breakpoints were determined by critical analysis of log2 CNV ratios and average CNV smooth signals. Random noise was extensive through the genome, giving CNVs that ranged a full log2 ratio point in some areas. It was found that by judging analysis by averages in CNVs gave increased resolution as to significant gains in CNVs. Average CNVs across the genome that represented no gains were detected at a copy number of 2. The average CNV values found in areas of gain were at 3. The average copy number threshold for significant gain was chosen at 2.5. The estimated range of a translocation was the average CNV increase from 2.0 to 2.5, with this area being preceded or followed by an area of average gain (CNV=3) and an area of average no-gain (CNV=2). These values were compared to log2 ratios of the same area to ascertain significance in average CNV values and resolve overly noisy areas.

Using sample 1, the translocation breakpoint of chromosome 1 was determined to be between 27,746,336 base pairs (bp) and 27,961,397 bp (measures number of base pairs from the proximal terminus of the telomere on the p-arm). This region spans approximately 215 kb, and covers the regions of three genes: WASF2 WAS protein family, member 2
(range: 27,732,126 – 27,816,669 bp); AHDC1 AT hook, DNA binding motif (range: 27,860,756 – 27,930,143 bp); and FGR Gardner-Rasheed feline sarcoma viral oncogene homolog (range: 27,938,800 – 27,961,727 bp). Figure 6 shows aCGH data of chromosome 1 from sample 1 interpreted by Affymetrix Chromosome Analysis Suite using log2 ratios and average CNVs. Figure 7 shows the same data at a magnified level, focusing on the rearrangement breakpoint. Microarray resolution of chromosome 1 was lower for sample 2, giving a translocation range of 27,628,344 – 27,976,636 bp, correlating with the range determined from sample 1. Figures 8 and 9 show aCGH data of chromosome 1 from sample 2.

The breakpoint for chromosome 3 was determined to range between 50,058,332 bp and 50,776,966 bp. This covers a relatively large range of base pairs (nearly 719 kb) because this particular region on chromosome 3 is spanned by only seven oligonucleotide probes. This low concentration of probes decreases significance of CNV averages in this range, thus broadening the translocation range. Twenty-three genes span this region: SEMA3F sema domain; GNAT1 guanine nucleotide binding protein; SLC38A3 solute carrier family 38, member 3; GNAI2 guanine nucleotide binding protein; SEMA3B sema domain; C3orf45 chromosome 3 open reading frame 45; IFRD2 interferon-related developmental regulator 2; HYAL3 hyaluronoglucosaminidase 3; NAT6 N-acetyltransferase 6; HYAL1 hyaluronoglucosaminidase 1; HYAL2 hyaluronoglucosaminidase 2; TUSC2 tumor suppressor candidate 2; RASSF1 Ras association domain family member 1; ZMYND10 zinc finger; NPRL nitrogen permease regulator-like 2; CYB561D2 cytochrome b-561 domain containing 2; TMEM115 transmembrane protein 115; CACNA2D2 calcium channel; C3orf18 chromosome 3 open reading frame; HEMK1 HemK methyltransferase family member 1; CISH cytokine inducible SH2-containing protein; MAPKAPK3 mitogen-activated protein kinase-activated protein kinase 3; DOCK3 dedicator of cytokinesis 3. Figures 10 and 11 show aCGH data of chromosome 3 from sample 2. Only sample 2 provided data for the breakpoints on chromosome 3.

The breakpoint on chromosome 6 was determined to exist between 34,789,895 bp and 34,790,679 bp. The resolution for this breakpoint was extremely high relative to the other two breakpoints on chromosomes 1 and 3 (ranging 0.8 kb). This breakpoint lands completely within the UHRF1 binding protein 1 (UHRF1BP1) gene (range: 34,759,794 – 34,845,291 bp). This is the only gene that was found to completely cover the span of the determined breakpoint. Only sample 1 provided data for the breakpoints on chromosome 6. Figures 12 and 13 show aCGH data of chromosome 6 from sample 1.
Figure 1: (Top) Metaphase spread of a white blood cell with a chromosome 1, 3, 6 translocation. Arrows point to the p-arms of chromosomes 1, 3, and 6. (Bottom) Chromosomal karyotype of the same abnormal metaphase. The right chromosomes of 1, 3, and 6 are abnormal.
Figure 2: (Top) Chromosomal metaphase of an abnormal cell prior to microdissection. (Bottom) Same metaphase spread after microdissection. Arrows point to where DNA material was physically removed. Non-chromosomal entities outside of the metaphase spread are random DNA fragments.
**Figure 3:** FISH images showing PCR product scraped via microdissection of chromosome 1. PCR product was tagged with Digoxigenin-11-dUTP and used as a probe against a normal metaphase. Green regions are PCR product tagged with Digoxigenin-11-dUTP and blue regions are normal metaphase chromosomes tagged with DAPI. Images on the right are the same FISH metaphases with inverted DAPI, allowing for visualization of low-resolution banding patterns. The circles in red indicate chromosome 1, and the circles in green indicate chromosome 6.
**Figure 4**: FISH images showing PCR product scraped via microdissection of chromosome 3. Green regions are PCR product tagged with Digoxigenin-11-dUTP and blue regions are normal metaphase chromosomes tagged with DAPI. Images on the right are the same FISH metaphases with inverted DAPI, allowing for visualization of low-resolution banding patterns. The circles in red indicate chromosome 1, and the circles in green indicate chromosome 3.
Figure 5: Ideograms of the three abnormal chromosomes. (Top) Normal 1, 3, and 6 chromosomes, highlighted regions represent area where translocation breakpoint occurred. (Bottom) Visualization of complex translocation.
Figure 6: (Top) Microarray log2 data from sample 1 across entire chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average CNV data from sample 1 across entire chromosome 1 given in a smooth signal. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
Figure 7: (Top) Microarray log2 data from sample 1, magnified image at breakpoint on chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average CNV data from sample 1, magnified image at breakpoint on chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
**Figure 8:** (Top) Microarray log2 data from sample 2 across entire chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average CVN data from sample 2 across entire chromosome 1 given in a smooth signal. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
**Figure 9:** (Top) Microarray log2 data from sample 2, magnified image at breakpoint on chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average CNV data from sample 2, magnified image at breakpoint on chromosome 1. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
**Figure 10:** (Top) Microarray log2 data from sample 2 across entire chromosome 3. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average log2 data from sample 2 across entire chromosome 3 given in a smooth signal. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
**Figure 11:** (Top) Microarray log2 data from sample 2, magnified image at breakpoint on chromosome 3. Breakpoint lies within the indicated region. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average log2 data from sample 2, magnified image at breakpoint on chromosome 3. Breakpoint lies within the indicated region. The x-axis represents length of chromosome and y-axis represents average CNVs.
Figure 12: (Top) Microarray log2 data from sample 1 across entire chromosome 6. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average log2 data from sample 1 across entire chromosome 6 given in a smooth signal. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
Figure 13: (Top) Microarray log2 data from sample 1, magnified image at breakpoint on chromosome 6. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents log2 ratio of CNVs. (Bottom) Average log2 data from sample 1, magnified image at breakpoint on chromosome 6. Arrow indicates breakpoint. The x-axis represents length of chromosome and y-axis represents average CNVs.
Discussion

Balanced rearrangements are often considered to have a high probability of being the initiating factor in chromosomal degenerative diseases [11]. Considering this point, along with the absence of any other cytogenetically visible aberrations, the complex 1-, 3-, 6- translocation observed in this study may disrupt genes critical in the initiation and development of CLL. Although several genes and chromosomal regions have been implicated as factors in CLL development [2], currently no direct combinations of genes have been found that explain its initiation.

Microarray analysis narrowed the translocation breakpoint on chromosome 1 to three closely spaced genes: WAS protein family, member 2 (WASF2); AT hook, DNA binding motif (AHDC1); and Gardner-Rasheed feline sarcoma viral oncogene homolog (FGR). WASF2 codes for a protein product that links actin filaments to receptor protein kinases [12]. This protein complex has been observed to participate in signaling involved in cell shape and cellular motility, and has been implicated on several occasions as a possible factor involved in cellular invasion and metastatic properties of cancer cells [13,14,15,16]. AHDC1 has been linked to the signaling involved in human embryonic stem cell growth and development [17]. There is substantial evidence that the FGR gene is linked to over-production of B-cells, described in detail below. The breakpoint on chromosome 3 was narrowed to a subsection of 3p21.3, a region containing twenty three genes. Although a specific gene is difficult to identify with the large size of the range, the 3p21.3 region itself has been known for its abundance of tumor suppressor genes (TSGs), and will also be discussed in detail below. The breakpoint landing in this particular region suggests the possibility of disruption of a vital tumor suppressor gene. The translocation breakpoint on chromosome 6 was narrowed to within a single gene: UHRF1 binding protein 1 (UHRF1BP1). UHRF1BP1 is a known locus that is mutated in systemic lupus erythematosus [18]. We were unable to identify any impact this gene may have towards the initiation or progression of cancer.

FGR proto-oncogene

FGR is a member of the src family of non-receptor tyrosine kinases. Src protein tyrosine kinases are signal transducer enzymes that initiate cellular functions via activation of specific proteins. Tyrosine kinases function by transferring a phosphate group from ATP to a target protein. Work done by Francis Peyton Rous showed in chickens that a virus was responsible for the development of neoplastic tumors, called fibrosarcoma. It was later demonstrated by J. Michael Bishop and Harold E. Varmus that this virus contained a mutated form of the src tyrosine kinase, which lacked an inhibitory phosphorylation site and therefore was constantly active rather than active only when signaled [19]. In humans, mutated src-tyrosine kinase has been implicated in the development of colon cancer [20] and some leukemias [21]. Both src and FGR have been shown to act as negative regulators of the tumor suppressor gene DOC-2/DAB2, which is linked to prostate cancer [22].
Additionally, FGR has been found to play a primary role in cell proliferation in a small number of cell types and tissues, notably fetal liver tissue [23]. The role protein tyrosine kinases play in cell signaling may be subject to neoplastic promotion when mutated.

The FGR gene has been linked to apoptosis function and regulation. The FGR protein tyrosine kinase has been observed to regulate apoptosis by cooperating in the activation of hematopoietic lineage cell-specific protein 1 (HS1) [24]. It has been demonstrated that uncontrolled phosphorylation of HS1 renders B-cells insensitive to apoptotic stimuli [25]. In normal expression of FGR in natural killer cells and macrophages, the Fas ligand responsible for signaled cell death is activated by FGR protein tyrosine kinase [26]. However, B-cells with over-expression of the FGR gene show CD95/fas mediated apoptosis signaling diminished due to ligation deregulation of the CDw150 cell surface receptor [26]. These two mechanisms for down-regulated apoptosis stimulus both occur specifically in B-cells, indicating the possibility of B-cell immortalization such as that seen in CLL.

In 1986, Cheah et al. demonstrated that the FGR gene was induced in B-lymphocytes infected with the Epstein Barr Virus (EBV) [27]. When B-cells are infected with EBV, the lymphocytes become immortalized as stable cell lines. Although the B-lymphocytes were immortalized, they did not acquire fully neoplastic properties, suggesting that FGR may induce a neoplastic state in conjunction with additional genetic mutations [27,28]. The precise mechanism as to why FGR is promoted by EBV infection is not known; however it was discovered the virus increased transcriptional rates on a promoter upstream of the normally transcribed promoter for FGR. The gene product was extended by an exon 0.3 kb longer than the standard FGR transcribed region. The functionalities of this extended gene product are unknown [23,29].

FGR has also been found to play a critical role in cell migration. In myeloid leukocytes, it participates in β2 integrin signaling [30,31]. This acts as a negative regulator within the signaling pathway inducing cellular motility, and can thus cause rapid cell shape change [32,33,34]. Deregulation of cellular mobility functions has been hypothesized as a step in the development of metastatic abilities of cancerous cells.

3p21.3 TSG cluster

The 3p21.3 region contains numerous TSGs that when deleted or mutated can supplement the progression of certain cancers. Ji et al. examined the region’s tumor suppressor ability by inactivating the entire region, causing abnormal functioning in a variety of cellular processes including cell proliferation, cell cycle kinetics, signaling transduction, ion exchange, and apoptosis. It was also demonstrated that when 3p21.3 was restored in tumor cells with this particular deletion, tumor growth was suppressed [35]. Several mechanisms employing different genes in the 3p21.3 region have also been proposed for
tumor development through loss of heterozygosity and damaged DNA mismatch repair mechanisms [36,37].

The slightly broader region of 3p21 is known to be a common fragile site and is highly prone to rearrangements [38]. Deletions and rearrangements of 3p21 have been observed in a variety of cancers, including acute lymphocytic leukemia (ALL) [39,40,41,42,43], chronic myeloid leukemia (CML) [38,39], acute myeloid leukemia (AML) [39,44], myelodysplastic syndrome (MDS) [39,44], mesothelioma and lung cancers [38,39,45], and in some treatment related leukemias [39,40,44]. One case study of a patient diagnosed with prolymphocytic leukemia demonstrated that when neoplastic cells were transformed with EBV, the cells experienced recurrent chromosomal aberrations at 3p21 [46]. Furthermore, cryptic losses of 3p21.3 have been detected in several CLL studies [47,48,49].

Out of the twenty three chromosomes that span the translocation region found in this study, thirteen are either TSGs or TSG candidates. Hesson et al. defined a minimal critical 120 kb region that lies within the bounds of our translocation range on 3p21.3 that is consistently lost in varying forms of cancer [50]. Within this region, the following eight genes are encoded and are each TSG candidates: HYAL2, TUSC2, RASSF1, ZMYND10, NPRL, CYB561D2, TMEM115, and CACNA2D2. Of these genes, RASSF1 and NPRL have been shown to disrupt DNA mismatch repair mechanisms when mutated [50,51,52] and HYAL2 produces a GPI-anchored cell surface protein that serves as a receptor for the oncogenic virus Jaagsiekte sheep retrovirus [50,52,53]. Of the TSGs located outside this critical range, but still within the translocation range are MAPKAPK3, CISH, HYAL1, HYAL3, and SEMA3B and are involved in cell differentiation, cytokine signaling, cell proliferation and differentiation, cell proliferation and differentiation, and apoptosis regulation, respectively [52].

Possible neoplasia mechanism

Due to the number of genes found within the translocation range of chromosomes 1 and 3, this study was unable to determine an exact genetic outcome produced by this translocation. However, by observing the genes within the ranges, we can infer possibilities as to the genetic effect of the rearrangement. The initiation and advancement of leukemia is considered to be due to two separate mutations, one affecting cell proliferation (class I mutation) and the other affecting cell differentiation and immortalization (class II mutation) [54]. The FGR proto-oncogene is a likely candidate for a class II mutation in CLL. Over-expression of tyrosine kinases is often thought to be a gain-of-function mutation, as the proteins are normally expressed in low levels and are often involved in cellular proliferation functions. Over-expression or mutations in protein tyrosine kinases can potentially deregulate cellular proliferation, acting as a class I mutation. However, FGR expression is only known to correlate with cellular proliferation in a small number cell types, implying that its over-expression or mutated protein product may not cause the production of acute
levels of neoplastic cells. This fits the description of standard CLL development, as it is the gradual and slow accumulation of immortalized B-cells in the body. Elevated levels of expression for an abnormal FGR gene product are found in immortalized B-cells infected with EBV. Additionally, FGR participates in anti-apoptotic functions, further increasing neoplastic characteristics in its over-expression. B-cell immortalization and deregulation of apoptosis further suggests FGR as a class II mutation.

A class I mutation may exist at the gene interrupted by the translocation on chromosome 3. The large concentration of TSGs at the 3p21.3 region gives a relatively high probability that a TSG is either broken by the translocation or expressed irregularly due to disruption of the promoter region or other regions regulating gene expression. TSGs are normally actively expressed genes, functioning in many cellular roles including DNA repair and regulation of proliferation. If a TSG is knocked out, cellular processes may lose levels of regulation associated with the TSG, effectively acting as a class I mutation.

To conclude, this study puts forth a possible genetic mechanism for the onset of CLL. The individual diagnosed with CLL had no other apparent aberrations besides the translocation in their chromosomes, suggesting that the rearrangement may have initiated the progression of CLL. Using aCGH analysis in conjunction with microdissection techniques allows for precision mapping of balanced translocations that would otherwise be undetectable through standard aCGH analysis. Through the use of selective aCGH, translocation breakpoints could be narrowed to a small margin and genes analyzed. A possible genetic cause employing a class I and class II mutation was extrapolated from the data obtained by aCGH. Results and previous studies suggest the respective class I and class II mutations in a TSG located in 3p21.3 and FGR may cause the development of neoplasia of human B-cells. Further studies with the use of zoomed-in microarray are currently being pursued with the purpose of determining with certainty the breakpoints of the translocation and eventually cloning the genes located at the breakpoints. This study represents a case study in which a genetic mutation may have caused CLL or very closely related symptoms, and does not imply that these genes are responsible for all cases of CLL or even a subset of the disease. Although data and gene function suggests a possible initiation mechanism, this study is considered preliminary, with further studies needed.
Literature Cited


