**MicroRNAs in Chronic Lymphocyte Leukemia**

**Key Words:**

**MicroRNA, genome sequencing, B cells**

**MicroRNAs can be used for gene and genome editing in medical practices. They can be used to help identify the differences in healthy B cells and B cells in individuals with Chronic Lymphocyte Leukemia (CLL). In this study, 94 patients with CLL were tested to see their level of expressions of the 70-kD zeta-associated protein (ZAP-70) and the immunoglobulin heavy-chain variable-region (IgVh) gene, compared to individuals who do not have CLL. Everyone in the study was on a schedule for their treatments, so the researchers took into consideration the time between their diagnosis and length of treatment. This study used genomic sequences to identify the differences in expression from the genes in each patient. The abnormalities were identified in 42 microRNAs, and 13 out of the 190 genes were able to express the difference between healthy B cells and B cells in individuals who have CLL. The microRNAs used in this study were used to help identify the presence or absence of progression from diagnosis to current treatment time. During the research, a germ-line mutation was identified as a deletion of a healthy allele, and this expression would be the cause of CLL. The researchers in this study went on to do the same genome sequencing on 160 patients who do not have CLL and do have a family history of CLL. These patient's panels came back to support the researcher's findings. The use of microRNAs and genome sequencing can be used to help identify mutations that express different diseases.**

**Introduction**

While Chronic Lymphocyte Leukemia (CLL) is the most prominent form of leukemia, there is not much information known about the cause and progression of the disease. Researchers have come to find that CLL is from a mutated B cell, and this study was conducted to find more information on the effected B cells. Aggressive forms of CLL have evidence of no correlation to mutations in the IgVh gene and high levels of ZAP-70. These are the only consistent findings of CLL.

The deletion of DNA in chromosome 13q13.4 is evident in roughly half of the patients diagnosed with CLL. The chance of this deletion increases as the time without treatment for CLL increases. Since the chromosome and DNA are deleted during the time of infection, many doctors and researchers believe CLL is pathogenic or obtains pathogenic characteristics.

MicroRNAs have a wide variety of shapes and sizes. Most microRNAs consist of 19-25 nucleotides. These nucleotides are from foldback structures of nearly 65-110 nucleotides. MicroRNAs are complex structures with a great variety in structure and function. While doctors and researchers have not found all microRNAs in the human body, roughly half of the known microRNAs are all found in the dominant cancer regions of genomes. The location of the microRNAs supports the hypothesis that they are within the pathogenic pathways for human cancers. The researchers used microchips to discover that CLL cells (CD5+ B cells) have different and more unique readings on the microchips compared to healthy CD5+ B cells. There were several tests with the microchips done on multiple samples from the patients with CLL to recognize patterns expressed by B cells in CLL patients compared to healthy B cells. Both patients with CLL and patients without CLL were tested the same to differentiate the findings.

**Recent Progress**

The microRNA microchips were used to recognize expressions and patterns from B cells in patients with CLL compared to patients who do not have CLL. This was used to narrow down the expressions that could be correlated with the cause and progression of CLL. The researchers in this study used a 20% expression of ZAP-70 as a cutoff for a positive test, while to determine the homology of the IgVh, 98% was the cutoff. The 94 patients in the study were narrowed down into 4 test groups. Group 1 consisted of 36 patients who had the expression of ZAP-70 and unmutated IgVh. Group 2 consisted of 10 patients with the expression of ZAP-70 and mutated IgVh. Group 3 consisted of 1 patient with no ZAP-70 expression and unmutated IgVh. Group 4 consisted of 47 patients with no ZAP-70 expression and mutated IgVh. These groups were tested with many procedures to verify the correct group to ensure the most accurate results. The researchers found 13 possible microRNAs that could differentiate groups 1 and 4 for further research since these groups had the most patients. The researchers then used a machine called The Support Vector Machine, which subsequently correctly determined each patient's classification among the groups. There was no expression from the microchip that was able to differentiate groups 2 and 3, so the researchers then split group 2 into groups 1 and 4.

The researchers started their study with the 50 known expressions of ZAP-70. They needed to be sure of the accuracy of The Support Vector Machine, so they tested the 50 patients, and the machine came back 100% in line with the original findings of the researchers. The microchip was proven to be able to differentiate similar genes and expressions. The doctors and researchers tested multiple similar genes with the microchip, and it was successful in determining the differences in function and expression each time.

During this study, 41 out of the 94 patients had started treatment for their CLL, according to The National Cancer Institute of Working Groups. The PAM survival analysis was used to determine the expressions of 190 microRNAs from the time of diagnosis to therapy. The PAM analysis was also used on the patients who had not started treatment, to express the changes over time without treatment compared to patients who had started their treatment. Each patient that was going through treatment had a different time between their procedures, so the researchers found nine microRNAs to use to determine the difference between those with long term treatments compared to those who had short term treatments.

Mutations are more common on genes that have an unordinary expression for that particular gene, rather than normal expressing genes since their function would be different. Germline and somatic mutations were the most common, consisting of 15% of the patients. 42 microRNAs were used and tested, and of these 42, five expressed mutations. These consisted of miR-16-1, miR-27b, miR-29b-2, miR-187, and miR-206. They tested these results on 160 patients who did not have CLL, to ensure the readings were not from another gene that wasn't accounted for. Even after testing the 160 patients without CLL, their tests were supported due to none of the mutations being expressed.

CLL has been thought to have a hereditary impact, so the researchers conducted a test to find similarities among family members. Eleven of the patients who had an abnormal microRNA expression, and 8 of them knew of a family member that also had been diagnosed with CLL previously. The abnormalities that were expressed were found in the RT-PCR region. The 11 patients were then determined to have a C to T homologous substitution in the pri-miR-16-1. The pri-miR-16-1 region is known for its importance in the genomic area of all primates.

The readings of the miR-16-1 in patients with CLL showed a low expression compared to healthy B cells and the deletion of the gene 13q14.3 in most of the CLL patients. These findings were not evident in the 160 control patients, which further supports their research. All 11 patients at the end of the study were determined to have a C to T homologous substitution. At the same time, the normal is heterozygous, which supports the hypothesis of CLL stemming from a germ-line mutation, and one of the 11 patients had a relative who was diagnosed with CLL and breast cancer, which also supports the hypothesis of a germ-line mutation.

**Discussion**

To conduct the most accurate research, the researchers and doctors considered many possible factors that could disrupt any results. They focus on the importance of considering the patients' time of diagnosis and ongoing treatment since the progression of cancer can slow down during treatment. The researchers found a significant correlation among many factors during this study. Doctors typically postpone treatment for CLL until it is readily developed. Still, if there is excellent evidence for CLL in a patient, treatment should begin as soon as possible to help prevent the rapid spreading of such a fatal disease and prevent any more mutations that could occur.

The functional significance of microRNAs and its effects on the pathogenic behavior of CLL has been supported heavily through this research. The evident similarities between 7 of the 13 expressions of the microRNAs are helpful to researchers to know what to look for when these expressions show up on lab results. These findings can help future research to identify similar aspects of other pathogenic diseases and how they progress through their cycle. Abnormal expressions identify in this study are shared amongst other common cancers.

MicroRNAs miR-16-1 and miR-15a are typically tumor suppressors, but since they are expressed abnormally, those individuals are more susceptible to mutations that lead to tumors. The homologous substitution and a germ-line mutation prevent the tumor suppressor gene- this is known as the Knudson model in many oncology studies. This study helped support the hypothesis that germ-line mutations and homologous C and T substitutions cause harmful diseases that can spread through family lines once the mutation occurs.

There were no abnormal expressions in the 160 patients that did not have CLL, which is essential because there were no abnormalities in any of their B cells, while those who had CLL had defects in all of their B cells as CLL progressed.

The mutations expressed in this study could be a fraction of the mutations that are present because the researchers only tested about 20% of known microRNAs to observe abnormalities. If this study was to continue with the rest of the microRNAs, more information could be given to correlate the causes and any other aligning information.

**References**

Callin, G. “A MicroRNA Signature Associated with Prognosis and Progression in Chronic Lymphocytic Leukemia.” NEJM. (2017, October 21).