The T-Cell Acute Lymphoblastic Leukemia 1 (TAL1) gene encodes for a transcription factor that is a major regulator in hematopoiesis. This transcription factor affects the differentiation of the Hematopoietic Stem Cell (HSC) into lymphoid cell lineages, specifically T lymphocytes. T lymphocytes play a major role in the body's cell-mediated immunity. Any defect in TAL1 causes an immunocompromised patient. There are several mutations that can cause defects in the T-Cell Acute Lymphoblastic Leukemia 1 gene. One specific mutation studied is a translocation, exchanging genetic information between chromosome 1 and chromosome 14. This translocation is specifically referred to as t(1p33;14q11). This translocation induces an overexpression in the TAL1 transcription factor. With this excess protein, tumor activity is created in the T lymphocyte development pathway, producing the T-cell Acute Lymphoblastic Leukemia (T-ALL) phenotype. There are many current therapies, like chemotherapy, that treat the symptomatic ailments of T-ALL, but are slow curative therapies. Gene therapy is a novel treatment that can be used to directly target the chromosomal mutations causing T-ALL. One of the major drawbacks of current gene therapy techniques is the lack of specificity seen in vectors used to target the chromosomal abnormalities. Improvements in gene therapy can be accomplished through the use of Zinc Finger Nucleases (ZFNs), a type of engineered nuclease. In theory, gene therapy using ZFNs suggests a realistic cure for T-ALL. This paper outlines the potential use of ZFNs in creating more effective gene therapy treatments for T-ALL patients with the t(1p33;14q11) mutation in TAL1.

Key words: gene therapy, ALL, zinc finger nucleases, hematology

Introduction

Hematopoiesis is the production of blood cells through the process of cellular development, differentiation and proliferation. As seen in Figure 1, all hematopoietic cells arise from a common pluripotential stem cell.1 After passing through the progenitor cell stage, each cell is then directed to the myeloid or lymphoid pathway. The immature cell then distinguishes further into the unipotential committed cells. Here the exact lineage of the cell is definite. After a series of further proliferation and maturation stages, mature blood cells are released into the peripheral blood to complete their function.2

Throughout this process, developing Hematopoietic Stem Cells are constantly influenced by cytokines and transcription factors. Therefore, expression and regulation of transcription factors and cytokines is critical in hematopoiesis. From the common lymphoid progenitor cell, IL-2 and IL-7 cytokines dictate the differentiation into the T lymphocyte cell lineage (T-cells). T-cells are one of the main cell lineages responsible for humoral...
immunity. There are many T lymphocyte subtypes, two of which are T helper cells (Th) and Cytotoxic T-cells (Tc). T helper cells work to activate other cell mediated immunity responders, such as antibody producing B cells. Cytotoxic T-cells use MHC class 1 specific binding properties to target antigenic molecules. By releasing their perforin, cytoxins, granzymes and granulysin, Cytotoxic T-cells induce apoptosis to foreign peptides.3

The T-Cell Acute Lymphoblastic Leukemia gene, also known as TAL1, encodes one transcription factor integral to Hematopoietic Stem Cell differentiation into the T-cell lineage. The protein product of the TAL1 gene, TAL1 transcription factor, is a major regulator of HSC differentiation.4 (1) With increased amounts of TAL1 transcription factor, the hematopoietic cell differentiation pathway is disturbed and only immature lymphoblast T-cells are produced.6 The over production of lymphoblastic cells leads to a malfunction in cellular immunity, as well as many other pathologic consequences. When lymphoblasts become over 25% of the cells found in a patient blood sample and contain cell surface markers specific to the T-cell lineage, the disease is known as T-cell Acute Lymphoblastic Leukemia (T-ALL).2 The first purpose of this paper is to investigate the TAL1 gene and its role in T-ALL disease progression.

There are several known mutations causing the T-ALL phenotype. One specifically is a translocation between chromosome 1 and 14.3 This translocation, t(1;14), occurs between TAL1 and the T-cell Receptor Alpha gene (TCR Alpha), causing an over expression in TAL1.5,6,7 There are current chemotherapies and radiation therapies available to treat T-ALL. These therapies offer assistance to patient recovery, but are neither highly efficient nor fast acting. Secondly, this paper aims to investigate new therapeutic options for T-ALL patients using improved gene therapy techniques.

Gene therapy is defined as the insertion, deletion or modification of defective genes within a genome to treat a disease. The delivery of DNA containing a gene of interest into a cell is completed by a process known as transfection.8 In 1990, the first case of gene therapy in history was completed at the National Institutes of Health in Bethesda, MD, United States of America. Two patients participated in the study, both having a rare form of severe combined immunodeficiency disease (SCID) called adenosine deaminase (ADA) deficiency.9 The treatment was deemed a success, although the results were only temporary and immune reactions to the vectors used was observed. This indicated that although the recombinant gene had successfully replaced the mutant gene, gene therapy had many pitfalls that needed to be addressed if it was going to be considered as a legitimate candidate for curing genetic disorders. Today, gene therapy has made significant progress with the mechanism of correcting defective genes to inhibit disease development, but gene therapy is still in its novice stages. Gene therapy has potential for widespread clinical use in disease such as T-ALL, but only if its imperfections are corrected. One-demise of gene therapy is insertional mutagenesis. Insertional mutagenesis is defined as the process of vectors inserting the recombinant DNA to off site locations in the genome, thus creating adverse effects for patients.

Zinc Finger Nucleases (ZFNs) are an artificial restriction enzyme that can greatly improve the specificity of gene therapy targeting the disease causing mutation. ZFNs combine a DNA-binding domain to a DNA-cleavage domain, producing an artificial restriction enzyme. The zinc finger DNA-binding domain can be modified to target specific DNA sequences within a genome. Zinc fingers each contain three nucleotides. A Zinc Finger Nuclease contain between three and six zinc fingers, therefore comprised of a nine to 18 base pair target sequences.10 The multitude of nucleotide combinations that can be created with in one ZFN structure theoretically allows for one specific locus to be targeted in the genome. Site-specific gene therapy using ZFNs will allow for more effective treatment and a possible cure for T-ALL patients. The role of TAL1 in T-ALL diseased phenotype expression and gene therapy using Zinc Finger Nucleases as a curative therapy for T-ALL is discussed throughout the remainder of this paper.
Discussion

T-Cell Acute Lymphoblastic Leukemia 1 (TAL1)

The T-Cell Acute Lymphoblastic Leukemia 1 (TAL1) gene encodes for a transcription factor that contributes to the transcriptional regulation of the Hematopoietic Stem Cell (HSC). TAL1 protein displays a basic helix-loop-helix (bHLH) domain necessary for early HSC development and differentiation. This tertiary protein motif displays a 331 amino acid structure. TAL1 can be found on human chromosome 1p32-33, denoting its location between the 32nd and 33rd position on the short arm of chromosome 1.6,11

TAL1 is highly conserved through evolutionarily related species. Gene conservation suggests the importance of this transcription factor in the proper proliferation of the Hematopoietic Stem Cell. Gene conservation correlates to a gene’s role in developing a fully functional organism. If a gene, like TAL1, is seen early in evolutionary history and conserved to more distant evolutionary descendants, it can be assumed that the gene is under selection pressure to maintain its integrity for future species. Figure 2 displays the conservation between five vertebrate loci; Human (H), Mouse (M), Chicken (C), Pufferfish (P) and Zebrafish (Z). TAL1 homology is depicted by the colored boxed, representing exon conservation. Note that the gene order between species is also conserved. The gray shading represents loci size difference between the five vertebrates. Variation in loci size is due to differing sizes of 3’ UTR.

Figure 2. TAL1 Homology Between Five Vertebrate Species12

TAL1 Translocation causing T-cell Acute Leukemia

As previously stated, TAL1 is involved with the transcriptional regulation of the Hematopoietic Stem Cell (HSC). The HSC is the pluripotential precursor for all blood cell lineages. Therefore integrity of the TAL1 is important for proper hematopoiesis, the formation and development of blood’s cellular components. With mutation of the TAL1 gene, deficiency in blood cell lineages occurs. Specifically, T lymphoid cells’ ability to differentiate into mature cell lines is compromised. TAL1 is a known proto-oncogene, or a gene that can be mutated to display up-regulated expression of its wild type protein, creating tumor activity in cells.2,4,6

TAL1 affects T lymphocyte cell lineages. A mutation in TAL1 may cause several abnormal pathologies by disrupting the HSC differentiation and maturation pathway. Disease progression of leukemia, like many other HSC disorders, begins with expansion of immature, malignant cells crowding the stroma of the bone marrow. This space is necessary for blood cell development. The lack of this environment for cell proliferation and differentiation prevents normal hematopoiesis. Eventually, the absence of stromal space causes abnormal, immature blood cells to diffuse out of the bone marrow into the peripheral blood and secondary tissues. Here begins the abnormal physiological process of leukemia.2

Leukemia is one of the major disease phenotypes associated with a mutation in TAL1. There are many divisions of leukemia, set in place by both the French-American-British (FAB) and the World Health Organization (WHO) guidelines. T-cell Acute Lymphoblastic Leukemia (T-ALL) is the most common disease profile associated with a mutation in TAL1. T-ALL is a cancer of the white blood cells, affecting the lymphoid differentiation pathway. T-ALL is characterized by excess lymphoblasts with T-cell Cluster Designation (CD) markers. Lymphoblast cells mark an immature lymphocyte, not intended for peripheral blood circulation.2,3
These malignant cells may be seen in the bone marrow, peripheral blood and secondary organs depending upon disease progression. General ALL is most common in childhood. T-ALL however, is prevalent in both pediatric and adult forms. T-ALL comprises 15% and 25% of childhood and adult ALL respectively.13

Patients presenting with T-ALL generally have high white blood cell counts (>100x10^9/L) and may display organomegaly, particularly mediastinal enlargement and CNS involvement.2,13 Diagnosis of T-ALL involves bone marrow sample analysis. As described by the WHO, 25% or greater of bone marrow cells must be lymphoblasts. T-ALL patients, however, most often display bone marrow aspirate of almost entirely of lymphoblasts at the time of diagnosis. The T-Lymphoblasts observed are often medium-sized with prominent nucleoli and moderate amounts of cytoplasm.2 Figure 3 denotes a typical bone marrow smear found at diagnosis. T-Lymphoblasts comprise most cells visible; taking on previously described common characteristics.14

Cytogenetic diagnostic tools show that chromosomal abnormalities occur in 50-70% of T-ALL patients.5 Cellular rearrangements that occur in TALL affect the production of a transcription factor protein that has vital functions in HSC differentiation, thus leading to T-ALL. Reciprocal translocations of the T-cell Receptor Alpha and TALL genes house one of the most common translocations causing the T-ALL phenotype. One chromosomal aberration involving TALL that causes T-ALL is a translocation between chromosome 1 at position 1p33, the TALL loci, and chromosome 14 at position 14q11, containing T-cell Receptor Alpha chain (TCR Alpha) loci. This specific rearrangement for TALL is abbreviated as t(1p33;14q11). As a consequence of the translocation, TALL is transposed from its normal position on chromosome 1 into the T-cell Receptor Alpha chain loci, found on chromosome 14.6,7,15

Ectopic expression is defined as the expression of a gene in an abnormal place in an organism. Ectopic expression of the TAL1 and the TCR Alpha gene occurs in 3% T-ALL patients. The malignant translocation places TAL1 under the TCR Alpha promoter, causing up-regulated expression of TAL1 compared to expression seen in a non-diseased individual. This specific rearrangement for TALL is abbreviated as t(1p33;14q11). As a consequence of the translocation, TALL is transposed from its normal position on chromosome 1 into the T-cell Receptor Alpha chain loci, found on chromosome 14.6,7,15

Studies have shown over expression of TAL1 in T-ALL patients.16 The over expression of a proto-oncogene, like TAL1, is a hallmark of malignant cancers. Elevated expression levels in T-ALL samples can be seen in Figure 4.17 The oncogene transcription factor TAL1 expression levels in both T-ALL and control patients are shown. Immature, cortical and mature thymocyte cell populations were extracted from bone marrow and blood samples of T-ALL patients and thymus tissue from control patients. Expression of TAL1 was examined at each thymocyte subtype through cDNA synthesis and QRT-PCR analysis. TAL1 expression was significantly up-regulated in all T-Cell phenotypic subgroups compared to the T-Cell stage specific control population. Thus supporting that increased, ectopic expression of TAL1 occurs in T-ALL patients with chromosomal aberrations.

The t(1p33;14q11) translocation provides a worse prognosis than many other genetic causes of T-ALL.7 Because this translocation causes such an aggressive phenotype in patients, it is important to identify the molecular area causing the disease to allow for more precise treatment. Either drugs or transgenic alteration methods can be used to target the specific molecular area that has undergone genetic mutation. By doing so, a more effec-
tive cure for T-ALL can be introduced in clinical medicine, surpassing the success of current available therapies.

This image displays oncogene transcription factor TAL1 expression levels in both T-ALL and control patients. Immature, cortical and mature thymocyte cell populations were extracted from bone marrow and blood samples of T-ALL patients and thymus tissue from control patients. Expression of TAL1 was examined at each thymocyte subtype through cDNA synthesis and QRT-PCR analysis. TAL1 expression was significantly upregulated in all T-Cell phenotypic subgroups compared to the T-Cell stage specific control population.

Figure 4. Elevated TAL1 Expression Levels seen in T-ALL Patients

Current treatment for acute leukemia consist of a combination of chemotherapy, steroids, radiation therapy, and if necessary bone marrow or stem cell transplants. These treatments provide slow recovery, putting patients on multiyear treatment protocols. The goal of these treatments is to have the patients enter a state of lasting remission. Lasting remission generally displays less than 5% blast cells in the bone marrow sample taken from patients. The earlier T-cell Acute Lymphocytic Leukemia is detected and eliminated from the bone marrow, the more effective the treatment. The multiyear protocol most T-ALL patients endure requires time to bring patients into remission. If a new therapy directly targets the genetic mutation causing T-ALL, malignant T-cells could more quickly and efficiently be eradicated from the body, allowing for a greater chance of overcoming the malignant cancer.

Gene Therapy using Zinc Finger Nucleases (ZFNs) for Curative T-ALL Treatment

Studies have previously shown the potential for translocations causing cancer phenotypes to be modified by experimental therapies. Gene therapy is an experimental therapy that holds a promising outlook for T-ALL patients suffering from chromosomal translocations. There are two distinct types of gene therapy: gametic gene therapy and somatic gene therapy. Gametic gene therapy directly introduces a new functional gene into a germ line cell, either a sperm or egg. The corrected gene is then embedded into the genome of the gametic cell. This causes a heritable change in the genome, and thus inheritance of the improved gene occurs. In theory, this is currently the most effective method in terminating the inheritance of genetic disorders, however, the highly controversial ethics surrounding gametic cell research has prevented this technique from becoming a full force gene therapy treatment. In contrast, somatic cell gene therapy is the current proposed treatment for genetic disorders in humans. Insertion of a therapeutic gene directly to a somatic cell localizes the treatment to only the patient; the modified gene will not be inherited by offspring in sequential generations. The long term effects of somatic cell gene therapy are not as dramatized as the results of gametic gene therapy, but neither are the controversies surrounding it, making somatic cell gene therapy the best candidate for treatment.

There are several techniques used for gene therapy, specifically ones utilizing modified DNA or siRNA. The delivery of DNA into a genome aims to counteract for a loss of function mutations in an individual. The DNA plasmid is inserted into the host cell nucleus where it can induce expression of the transfected gene, allowing for compensation of the disease causing mutation. siRNA, however, can be used to affectively treat gain of function mutations. When present in a host cell, siRNA meets the mRNA transcript in the cytoplasm of the cell directly following DNA transcription and quickly induces degradation of the mRNA transcript, thus silencing the disease-causing gene. Each technique has a specific role that is dependent on the targeted disease. Gene therapy is a quality candidate to cure translocation-based diseases. Due to the translocation mutation seen in T-ALL, a combination therapy of non-traditional gene silencing without the use of siRNA and the insertion of recombinant DNA to replace the original functional genes would be used. This simplistic genetic change would allow for mutation specific therapy to be tailored to individual subtypes of T-ALL, like the t(1;14) mutation discussed.

Currently, the most common vectors used for gene therapy are viral vectors. Viral vectors utilize the viral replication cycle to integrate both their genetic material and the transfected genetic material into a host cell. Viral vectors should be used with caution, immune response to
the viral vectors and the short-lived nature of the gene therapy induced contribute to the demise of this treatment. Therefore, *In vivo* use is not always practical with viral vectors.  

Commonly, retroviral vectors are used to correct genetic abnormalities through gene therapy. Clinical strengths of the retrovirus are that they easily undergo genome integration, and therefore display sustained gene expression. Characteristics retarding the effective *in vivo* use of the retrovirus include their restricted ability to integrate into specific cell types. A secondary concern of retroviruses includes their common practice of integrating itself into random orientations of the host genome, a process known as insertional mutagenesis. Retroviral vectors are continually being improved to counteract their shortcomings. However, another innovative way to induce more effective gene therapy is to use a more effective vector.

This paper presents a novel way to prevent insertional mutagenesis caused by gene therapy viral vectors. Genome Editing with Engineered Nucleases (GEEN) provides an innovative approach to gene therapy by using site-specific engineered nuclease. One type of engineered nucleases is Zinc Finger Nucleases (ZFNs). ZFNs are artificial restriction enzymes, which are constructed by the joining of a zinc finger DNA-binding domain with a DNA-cleavage domain. The most commonly used DNA cleavage domain in ZFNs is the restriction enzyme FokI. The zinc finger DNA-binding domain can be modified through molecular techniques to target specific DNA sequences. A zinc finger has three nucleotides available for DNA binding. A Zinc Finger Nucleases contain between three and six zinc fingers, therefore each comprised of nine to 18 base pairs. A site-specific double strand breaks in the host genome is caused through the dimerization of two ZFNs. The use of two ZFNs allows for the targeted gene to be corrected at a pre-selected chromosomal site only through modifications of their DNA binding domains. Thus the complex structure of the ZFN theoretically offers the ability to target one specific locus in the genome for gene therapy.

Through the uses of site-specific ZFNs, the specificity of the gene therapy can be improved, preventing insertional mutagenesis. ZFNs have endless potential. Being a concept that was introduced only in the past decade, there are many facets of ZFN activity that may provide successful enhancement to gene therapy. Therefore, ZFN would allow more efficient introduction of the corrected *TAL1* gene into the target HSC, and prevent the over production of the *TAL1* protein associated with tumor expression in T-ALL.

Gene therapy correcting the defective gene through the use of ZFN artificial restriction enzymes provides a valid hope for curatively treating disease caused by the single t(1;14) mutation in the *TAL1* gene. ZFNs are capable of disabling an allele through Non-Homologous End Joining (NHEJ) or editing a defective allele through Homologous Recombination (HR). To correct the t(1;14) mutation seen in T-ALL, ZFNs coupled with wild type recombinant DNA would be used to induce allele editing of *TAL1*.  

The translocation t(1;14) seen in T-ALL is a simple and effective target for gene therapy. Therapy methods would include two ZFN specific sequences: one ZFN sequence specific for 1p33, targeting the *TAL1* gene, and one ZFN sequence specific for 14q11, targeting the TCR Alpha gene. The first ZFN specific for chromosome 1, would first be used to silence the translocated TCR Alpha DNA located at 1p33 after the mutation. Through the dimerization of the two ZFNs, the FokI restriction enzyme would induce a double strand break in the translocated TCR Alpha loci, disrupting its coding sequence. Then, the necessary portion of the wild type *TAL1* allele would be inserted into the cleavage sight and incorporated into the host genome through Homologous Recombination. This would ultimately disable the TCR Alpha allele and replace the *TAL1* allele under the correct promoter. A ZFN specific for 14q11 would then be used to silence the translocated *TAL1* DNA present on chromosome 14 after the mutation. Through first inducing a double stranded break in the translocated *TAL1* loci and then inserting the wild type TCR Alpha gene into the host genome through homologous recombination, a complete correction of the translocation mutation may occur. This will ultimately restore both alleles under their correct promoters, therefore decreasing over expression of *TAL1* and limiting tumor activity within the T-cell lineage.

The effective nature of gene therapy treating T-ALL is due to its residual effects on the Hematopoietic Stem Cell (HSC). The translocation mutation directly causes the over expression of *TAL1*, leading to deficiency in the HSC. By decreasing the expression of *TAL1*, correction of HSC proliferation and differentiation is possible. Because the HSC has proliferative and self-renewing capabilities the effects of gene therapy targeting the HSC population will become permanent. This evades one of the major demises of gene therapy: short lived treatment. Stem cells’ ability to self-renew decrease the need of repeated gene therapy administrations, or possibly even eliminates them. Gene therapy using ZFNs allowing...
site-specific editing of the chromosomal translocation causing the T-ALL phenotype would therefore be a long lasting, effective form of treatment. Because gene therapy targeting the HSC can be delivered in a limited or single dose to cure T-ALL, if clinically effective, it would be the most precise and immediate treatment option compared to current therapies. The use of ZFNs would also offer reduced side effects in contrast to traditional chemotheraphy or radiation therapy, providing a limited number of toxic exposures to the body. In closing, the proposed gene therapy treatment using ZFN specific proteins offers a serious hope for T-ALL patients in search for a cure to their ever present malady.

**Conclusions**

Hematopoiesis is tightly regulated by many cytokines and transcription factors, all involved in the development and differentiation of the Hematopoietic Stem Cell (HSC). The transcription factor encoded by the T-Cell Acute Lymphoblastic Leukemia 1 gene (TAL1) is of particular importance in the regulation of the HSC. As suggested in the name, TAL1 is involved with the presentation of many hematological disorders that affect the differentiation pathway of the HSC. It is known that the translocation exchanging genetic information between the TAL1 loci and the T-cell Receptor Alpha (TCR Alpha) loci creates a phenotypic expression of T-cell Acute Leukemia (T-ALL). T-ALL is a disorder specifically affecting the differentiation and development of T lymphocytes. When this translocation occurs, ectopic expression of the T-Cell Acute Lymphoblastic Leukemia 1 gene occurs. This over expression causes a disruption in the T lymphocyte differentiation pathway, producing only lymphoblast cells and tumor activity. These lymphoblasts inundate the bone marrow, impairing proper hematopoiesis. Without proper production of T lymphocytes, the body’s immune system is rendered insufficient. Current treatments of T-ALL focus on symptomatic management of the disease and do not offer a rapid and efficient method for curative therapy. Improved gene therapy methods offer a valid alternative for curing T-ALL more competently than existing available therapies.

Gene therapy is a novel type of treatment that can be used to directly target the chromosomal mutations causing T-ALL. Gene therapy’s efficiency can be improved through the use of Zinc Finger Nucleases (ZFNs). ZFN DNA binding domain can be artificially altered to increase genome-editing precision. By creating variable ZFNs specific to the loci of the target mutation, insertional mutagenesis can be prevented. In theory, because gene therapy treating T-ALL targets the development pathway of the HSC, this therapy would be efficient with limited exposure to treatment due to the proliferative and self-renewing nature of the stem cell. Gene therapy using ZFNs provides a logical way to cure T-ALL, improving the patient quality of life.

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**Figure 2. TAL1 Homology Between Five Vertebrate Species**

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**Figure 4. Elevated TAL1 Expression Levels seen in T-ALL Patients**

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References