REGULATION OF GENE EXPRESSION IN ACTUE LYMPHOBLASTIC LEUKEMIA BY IKAROS TUMOR SUPPRESSOR GENE

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most frequent cause of childhood malignancy. In the United States alone, during this year, over 3,000 children and adolescents will be diagnosed with ALL. The therapy for ALL has dramatically improved over the last 50 years, which results in an overall survival rate of 90% in children. However, the survival rate for the relapsed leukemia has not changed over the last 30 years and has remained at 50%. Thus, there is a need to develop a novel treatment approach to ALL.

Ikaros, a DNA binding protein, functions as a tumor suppressor in pediatric and adult ALL, and impaired Ikaros activity is associated with the development of a high-risk ALL. It is therefore imperative to understand how Ikaros regulates cellular proliferation in ALL in order to design a targeted treatment for this disease.

Previous studies have shown that inhibition of Casein Kinase 2 (CK2) results in the suppression of leukemia in cases of B cell ALL (B-ALL). Target therapies that employ CK2 inhibitors have been successful in regulating and controlling the epigenetic signature of B-ALL by exploiting CK2’s effect on Ikaros. The previous studies have thus provided a rationale to test the effect of CK2 inhibition on Ikaros in T cell ALL. The hypotheses that Ikaros represses transcription of the genes that are essential for the cell cycle progression in T-ALL, and that inhibition of CK2 in T-ALL enhances Ikaros-mediated regulation of the cell cycle progression resulting in a cytotoxic effect on T-ALL cells were tested. Results of the conducted experiments demonstrated that Ikaros represses expression of Cell Division Controller gene 2 (CDC2) and Cell Division Cycle
gene 7 (CDC7) in T-cell leukemia. CK2 impairs Ikaros function, whereas its inhibition restores Ikaros function, providing evidence that supports the use of CK2 specific target therapy for T-ALL.
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ACKNOWLEDGEMENTS

This research paper is made possible through the help and support from several individuals including my family, friends, and advisors. First and foremost, I would like to sincerely thank Dr. Sinisa Dovat, my thesis supervisor, for providing invaluable guidance and mentorship throughout the scientific process and for allowing me to use the materials and equipment the laboratory at the Penn State Hershey, College of Medicine, Department of Hematology/Oncology. I would also like to thank Dr. Chandrika Gowda, Dr. Mary McGrath and Mr. Jonathan Payne, for assisting me with all aspects of the experimental work for my thesis.

I extend my sincere gratitude to Dr. Sairam Rudrabhatla, my faculty advisor, for his constant support and for always keeping me on track with my deadlines and academic and extra-curricular work. Lastly, I would like to thank Dr. Omid Ansary, Senior Associate Dean, and Dr. Rafic Bachnak, Director for the School of Science, Engineering and Technology (SSET), for providing their support to Schreyer Honor Scholars like me at Penn State Harrisburg to continue our journey to achieve the highest scholarship possible in our field of interest and expertise.
Chapter 1: Introduction

Leukemia, commonly known as blood cancer, is scientifically defined as the cancer of blood-forming tissues. This cancer affects the lives of approximately 333,975 Americans annually, with an estimated survival rate of only 50%.\(^7\) The different types of leukemia can be broadly classified as acute or chronic, based on their rate of progression. Acute leukemia can be further divided into two types- lymphoid and myeloid, based on the lineage of the affected cells.\(^7\)

Acute Lymphoblastic Leukemia (ALL), also known as acute lymphocytic leukemia or acute lymphoid leukemia is a cancer of the lymphoblast cells- the body’s early immune cells. This malignancy is the most common type of pediatric cancer, with about three quarters of the total childhood cancer patients today are diagnosed with ALL.\(^7\)

Since ALL is the most common type of malignancy in pediatrics, it is also the most common cause of death in children among all types of pediatric cancers. The therapy for ALL has dramatically improved over the last 50 years, which has effectively increased the overall survival rate to 90% in children. However, ALL in adults is more lethal, with a survival rate of only 45%.\(^8,10\) In addition, the survival rate for the relapsed leukemia has not changed over the last 30 years and remains at 50%.\(^10\) Thus, there is a need to develop a novel treatment approach to ALL.

The current treatments are associated with severe side effects and significant morbidity, both during treatment and following completion of the treatment. The novel treatment should ideally be targeted, with few side effects. In order to accomplish this,
more insight regarding the regulatory mechanisms that control cellular proliferation in ALL is necessary.

1.1. Causes of Acute Lymphoblastic Leukemia

Acute Lymphoblastic Leukemia (ALL) is a disease characterized by uncontrolled division and proliferation of cells that serve as precursors to lymphoid cells. These cells are found in the bone marrow of humans. There are a few ALL cases that have been correlated with Down’s Syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia and Nijmegen breakdown syndrome. Some other epigenetic (environmental) factors that predispose individuals to ALL include exposure to ionizing radiation, pesticides, certain solvents or viruses such as Epstein-Barr Virus (EBV) and Human Immunodeficiency Virus (HIV). However, like most cancers, there is no definite knowledge of any specific diseases or conditions that could indicate the occurrence of ALL and a large majority is attributed to de novo (novel) mutations and consequent malignancies in otherwise healthy individuals.

1.1.1. Casein Kinase 2

Casein Kinase 2 (CK2), also known as protein kinase CK2, is an enzyme that belongs to the broad family of transferases. Its specific function is to transfer a phosphate group from one moiety to another, giving it the designation of a ‘kinase’. CK2 is a kinase that phosphorylates the amino acids serine and/or threonine, that have a hydroxyl group (-OH) in their open chain. This enzyme has a structure that is composed of four subunits, forming a tetramer of which two subunits perform a catalytic function
whereas the remaining two are regulatory in nature.\textsuperscript{4,15} Casein Kinase 2 has numerous substrates that it is able to phosphorylate, including compounds that serve as transcription factors that affect the production of RNA from DNA, oncogenes that play a role in the manifestation of cancer and tumor suppressors that control unnecessary proliferation of dividing cells.

Evidence has suggested that tight regulation of CK2 in normal cells is imperative-increased levels of CK2 have been noted in a variety of cancer cases.\textsuperscript{15,19} Furthermore, studies have shown that the cancer pathobiology might also be related to the deregulation of Casein Kinase 2. Finally, recent research has proven that Ikaros is one of the targets of CK2, providing for the rationale to study its effect in B and T cell ALL.\textsuperscript{19}
Chapter 2: Literature Review

2.1. Importance of Ikaros

The Ikaros gene (IKZF1) is one whose alterations have been associated with development of high risk ALL. This gene encodes a zinc finger binding protein, which acts as a master controller for differentiation of blood cells\textsuperscript{12,14}. Clinically, Ikaros has been found to be one of the most relevant tumor suppressors in ALL, and restoration of Ikaros activity has been suggested as a new and promising ALL therapy.

Of the pediatric ALL cases, 15% show either the deletion of one allele (copy) of Ikaros or a mutation in one of the two Ikaros alleles\textsuperscript{11}. This condition, known as haploinsufficiency leads to the production of 50% less protein product, along with an inactive Ikaros product, which could function as a dominant negative, leading to ALL\textsuperscript{14}. IKZF1 (Ikaros) mutation and deletion has been linked to Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML), further stressing the importance of Ikaros as the tumor suppressor.

2.1.1. Function of Ikaros in Leukemia

Ikaros, a DNA binding zinc finger protein is encoded by the gene IKZF1. In a normal cell, Ikaros functions as a tumor suppressor and is the master controller of B and T cell differentiation\textsuperscript{3,7}. Ikaros is thus a potent regulator of hematopoiesis, as well as the differentiation and proliferation of cells of lymphoid origin (lymphatic cells). As a DNA binding protein, Ikaros binds to specific parts of the genome to regulate the formation of RNA, a process known as transcription. Transcription is based, in part, on the structure of
the DNA that is being read or transcribed. DNA is typically found in the cell structurally like a bundle of “beads on a string”, where DNA strands are wound tightly around histone proteins that allow for the compact packing of DNA in the nucleus as chromatin. However, this packed (tightly wound) state of DNA is not conducive to transcription. Therefore, the packing and unpacking of DNA can be regulated to in turn regulate the transcription of genes. Ikaros utilizes epigenetic mechanisms such as the processes of acetylation and deacetylation to regulate gene expression.

In B-ALL, Ikaros has been shown to recruit histone deacetylases HDAC1 and HDAC2 to modulate gene transcription and thus functions as a transcriptional suppressor. Thus, in B cell-ALL, Ikaros regulates the expression of genes that are essential for cell cycle progression. Ikaros regulates gene expression via chromatin remodeling, by modifying heterochromatin to alter the accessibility of genomic DNA, which is directly used to regulate gene expression. Oncogenic protein Casein Kinase 2 (CK2) is a known post translational regulator of Ikaros, that inhibits Ikaros function in B-ALL. Thus, targeting CK2 with a specific inhibitor restores Ikaros function in B-ALL and achieves a therapeutic effect.

Preliminary experiments have also shown that Ikaros regulates key genes that are responsible for the differentiation of T cells. Lack of Ikaros has been shown to lead to impaired T cell differentiation, thereby confirming its role in T cell development. Ikaros also plays a role in the expression of CD4, CD8 (T cell surface proteins) and interleukin-2 or IL-2 (immune signaling molecule), which further confirms its function of T cell differentiation. However, the signaling pathways that Ikaros employs to mediate cell cycle progression in patients with T-ALL are currently unknown. It is therefore
imperative to study the role of Ikaros in T-ALL and the subsequent effect of CK2 on Ikaros as a therapeutic strategy.

2.2. T-ALL and current therapies

The current therapy of T-ALL includes a combination of cytotoxic chemotherapy drugs that have high toxicity and the treatment for T-ALL is associated with high morbidity of the patients. Despite this treatment, some of the patients with T-ALL are known to relapse. Treatment of relapsed T-ALL, requires high intensity chemotherapy, which is associated with life-threatening medical complications. Despite this treatment, survival of patients with relapsed T-ALL is below 50%. Thus, novel treatment modalities are necessary in order to improve survival rates for patients with T-ALL.

2.3. Specific aims for the regulation of Ikaros on T-ALL

This thesis tested the hypotheses that Ikaros represses transcription of genes that are essential for the cell cycle progression in T-ALL, and that inhibition of CK2 in T-ALL enhances Ikaros-mediated regulation of the cell cycle progression resulting in a cytotoxic effect on T-ALL cells.

This hypothesis was tested with the following specific aims:

Specific Aim #1: Analysis of expression of the genes that are essential for the cell cycle progression in T-ALL cells that overexpress Ikaros;

Specific Aim #2: Determination of the expression of the genes that are essential to the cell cycle progression in T-ALL cells that have Ikaros knocked out;

Specific Aim #3: Analysis of the effect of CK2 inhibition on the expression of genes that
are essential for the cell cycle progression and survival of T-ALL cells.
Chapter 3: Materials and Methods

3.1. Data sources

The study was performed in CCRF-CEM cells. They are lymphoblast cells from peripheral blood tissue from a female child with T cell – Acute Lymphoblastic Leukemia disease. These cells are suspension cells, and thus can be used in vitro studies without needing to bind to an adherent surface. They are tumorigenic, and can lead to the tumor phenotype in experimental studies, thus making them a suitable cell line for studying T-ALL. The experiments were performed in triplicates, and the results are presented as mean values with standard deviations. This is a standard experimental approach for these experiments that is used in molecular biology laboratories.

The completion of the thesis project required the use of biohazardous material. The use of these was approved by Biosafety Committee - Protocol number: SXD16-01-2.5 Title: Biological role of Ikaros and other transcription factors in leukemia. Approved date: 11/9/16.

3.2. Data collection

The aims of this proposed study were accomplished by using several molecular and cellular biology techniques that include quantitative real-time PCR (qRT-PCR), tissue culture, retroviral production, retroviral transduction and RNA isolation.

3.2.1 Cell Culture and other Reagents

For the experimentation process, cell cultures that were obtained from American Type Culture Collection (ATCC) were cultured in RPMI 1640 medium, with an
additional supplement of 10% of fetal bovine serum obtained from the company Hyclone. The cells in culture were then incubated at 37°C in a humid atmosphere with 5% carbon dioxide. The sequence of the full length Ikaros gene or the knockdown gene were confirmed using sequencing.

3.2.2 Quantitative RT-PCR

Quantitative RT PCR (qRT-PCR) is a technique that allows for the detection and consequent quantification of amplified product of RNA per replication/amplification cycle. The qRT PCR facilitated the quantification of target genes of Ikaros that have been discovered in previous studies via qChIP. This provided a confirmation for the rationale presented, and also a confirmation to proceed with the study of the effects of Ikaros repression. Thus, aim #1 was tested by qRT PCR technique.

The qRT PCR was performed as follows: The cells that were in culture were using to perform an RNA isolation process to isolate the total RNA of the cells that overexpress Ikaros and that have an Ikaros knockdown, using the RNeasy minikit by Qiagen. For this process, 1 μg of the extracted RNA was then put through a reverse transcription process, a technique that allows for the generation of a cDNA molecule from the RNA. This was done using the SuperScript™ first strand synthesis system by Invitrogen. The cDNA generated was then used to perform the qRT-PCR using the PerfeCta™ SYBR Green FastMix by Quanta Biosciences and was carried out in the StepOne Plus real time PCR system manufactured by Applied Biosystems. The qRT-PCR was performed with triplicates of each sample and was normalized using a standard 18S RNA. The data for the qRT-PCR was presented in the form of fold change as compared to the control cells.
3.2.3. Generation of Ikaros overexpression using retroviral transduction

Retroviral transduction system exploits the machinery of infectious viruses to integrate a novel gene that is a part of the viral genome into a host chromosome. The human IKZF1 gene was cloned into a bicistronic retroviral vector that expresses Green Fluorescent Protein (GFP). Such vector expressed human IKZF1 gene and GFP as a marker, under the control of long terminal repeats (LTR) in retroviral vector. Retroviruses were produced in Phoenix cells following transfection with retroviral construct. Retroviral transduction of CCRF-CEM cells was performed by spin inoculation protocol. CCRF-CEM cells that expressed fluoresced green at about 70-80% efficiency were then used for RNA isolation. The overexpression of Ikaros, as well as expression of Ikaros target genes was confirmed using qRT-PCR.15

3.2.4. Generation of Ikaros knockdown using Retroviral transduction

Retroviral transduction with retrovirus that contains Ikaros shRNA was implemented to suppress Ikaros production and thus tested the proposed aim #2. Ikaros function and mechanism in regulating expression of genes that are essential for the cell cycle in T-ALL were analyzed.

The retroviral transduction for the Ikaros shRNA knockdown was performed as follows: Unique shRNA human constructs for IKZF1 with GFP vector (pGFP-V-RS) were purchased from Origene. These constructs were cloned into retroviral vectors that were used to transduce CCRF-CEM cells to knock-out Ikaros. The retrovirus that
contains Ikaros shRNA was produced using producer cells. The CCRF-CEM cells were transduced with in a 24-well plate, using the retroviral transduction spin protocol. The shRNA were tested first, and the best gene knockdown shRNA for Ikaros was selected for further studies. The CCRF-CEM cells were transduced with the virus that contained this selected plasmid. Three days following transduction, the CCRF-CEM cells that expressed fluoresced green at about 70-80% efficiency were then used for RNA isolation. The knockdown of Ikaros, as well as expression of Ikaros target genes was confirmed using qRT-PCR.

3.2.5 CK2 shRNA Knockdown

Unique shRNA human constructs for CK2 alpha catalytic subunit with GFP vector (pGFP-V-RS) were purchased from Origene. These constructs were cloned into retroviral vectors that were used to transduce CCRF-CEM cells to knock-out CK2 alpha catalytic subunit. The retrovirus that contains CK2 alpha shRNA was produced using producer cells. The CCRF-CEM cells were transduced with in a 24-well plate, using the retroviral transduction spin protocol. The shRNA were tested first, and the best gene knockdown shRNA for CK2 alpha was selected for further studies. The CCRF-CEM cells were transduced with the virus that contained this selected plasmid. Three days following transduction, the CCRF-CEM cells that expressed fluoresced green at about 70-80% efficiency were then used for RNA isolation. The knockdown of CK2 alpha, as well as expression of Ikaros target genes was confirmed using qRT-PCR.
Chapter 4: Results

4.1. Results of experiments proposed for Aim #1

The overexpression of Ikaros via retroviral transduction in CCRF-CEM cells was confirmed by qRT-PCR. The retroviral transduction of Ikaros into CCRF-CEM cells resulted in 3-fold overexpression of Ikaros, as compared to the negative control that was transduced with an empty vector (This data is not shown, however experiments were performed for confirmation before the qRT-PCR process). Ikaros overexpression in CCRF-CEM cells resulted in reduced transcription of two genes that are essential for the cell cycle progression – CDC7 and CDC2 (This data is represented in Figure. 1) Both of these genes were shown previously to be directly regulated by Ikaros in leukemia.

4.2. Results of experiments proposed for Aim #2

Ikaros knockdown resulted in 2-fold reduced expression of Ikaros in CCRF-CEM cells, as compared with the negative control that contained scramble shRNA, as evidenced by qRT-PCR (This data is not shown, however experiments were performed for confirmation before the qRT-PCR process). Reduced expression of Ikaros in CCRF-CEM cells, resulted in increased expression of Cell Division Cycle gene 7 (CDC7) and Cell Division Controller gene 2(CDC2) in CCRF-CEM cells, as evidenced by qRT-PCR (This data is represented in Figure. 2). Together, results of experiments proposed in Aim #1 and Aim #2 suggest that Ikaros functions as a transcriptional repressor of CDC2 and CDC7 genes in CCRF-CEM cells.
4.3. Results of experiments proposed for Aim #3

Knockdown of CK2 alpha catalytic subunit in CCRF-CEM cells via retroviral transduction, resulted in reduced expression of CK2 alpha by 3-fold (data not shown). Reduced expression of CK2alpha resulted in decreased expression of CDC2 and CDC7 genes in CCRF-CEM cells, as evidenced by qRT-PCR (This data is represented in Figure 3). These data suggest that CK2 interferes with the ability of Ikaros to repress its target genes – CDC2 and CDC7 in CCRF-CEM cells.
Chapter 5: Discussion

Ikaros functions as a transcriptional regulator of gene expression. Studies in B-cell acute lymphoblastic leukemia (B-ALL) demonstrated that Ikaros can control cell cycle progression by directly regulating transcription of several genes that are essential for the cell cycle progression. The experiments performed in this study, showed that in T-ALL, Ikaros functions as a repressor of two genes that are essential for the cell cycle progression – CDC2 and CDC7. This was demonstrated using both gain-of-function (with Ikaros overexpression) and loss-of-function (with Ikaros knockdown) experimental approaches. Results of both experiments support the hypothesis that Ikaros directly represses transcription of CDC2 and CDC7 in T-ALL.

5.1. Cell cycle phases

The fate of cells is the body is known to be governed by a regulatory clock, that is in turn regulated by a highly-controlled group of signals and proteins. Dividing cells progress through a cyclic path called the cell cycle, and undergo changes that are divided into a series of 4 distinct phases, each of which is regulated by checkpoints.\(^{21}\) The first phase, known as G\(_1\) (growth phase 1 or gap phase 1) witnesses the preparation of a cell that is ready to undergo division. The cell checks for the sufficiency of nutrients, signs of DNA damage and extra cellular signals to proceed from G1 into the second phase- S. DNA replication or duplication occurs during the S phase (synthesis phase). The cell then progresses into the G\(_2\) phase (growth phase 2 or gap phase 2), where the cell checks for any errors that might have occurred during DNA replication and ensures the presence of appropriate levels of proteins required for the cell division. Finally, the cells enters the M
phase (mitosis phase) where it undergoes nuclear and cytoplasmic division. The M phase is further divided into four sub phases- prophase, metaphase, anaphase and telophase which result in nuclear division and then culminate into the last step of cell division which is cytoplasmic division.\textsuperscript{21}

5.2. Cell cycle regulation and cyclins

The cell cycle must be regulated to prevent any form of malfunction that could occur due to its complex nature.\textsuperscript{21} The regulation of the cell cycle can be broadly explained in terms of checkpoints, that are located at the transition state between two phases to ensure a smooth passover and prevent deregulation. Checkpoints look for DNA damage and block mutated cells from entering into the division phase.\textsuperscript{22} Checkpoints are also regulated by groups of proteins called cyclins and cyclin dependent kinases, whose levels vary during the different phases of the cell cycle.

5.3. G\textsubscript{1}-S checkpoint- CDC2 and CDC7

The G\textsubscript{1}-S checkpoint is the first checkpoint a cell will encounter when it begins its journey through the course of division. This checkpoint is governed by multiple cyclins and cyclin dependent kinases, of which CDC2 and CDC7 are essential for cell cycle progression.\textsuperscript{22}

CDC2, also known as CDK1 or Cell Division Controller 2, is a protein that belongs to the Serine/Threonine kinase family. It forms the catalytic subunit of a complex known as the M-phase Promoting Factor (MPF), that is critical for a cell to progress through the checkpoint G\textsubscript{1}-S and G\textsubscript{2}-M.\textsuperscript{20,22} The MPF complex facilitates the stabilization of proteins involved in mitosis. Thus, the catalytic subunit that CDC2 provides for in the
MPF plays a key role in cell cycle control.

CDC7, also known as Cell Division Cycle 7, is a protein that functions as a kinase and regulates transcription factors like E2F that regulate the transcription of DNA. Thus, due to the changes that occur in a cell during the G1 and S phase, CDC7 is an integral part of the regulation involved in the cell cycle.

5.4. CK2 and regulation of IKAROS, CDC2 and CDC7

CK2 is an oncogenic kinase, which is overexpressed in T-ALL. It has been shown that in B-ALL, CK2 inhibits the ability of Ikaros to function as transcriptional regulator, by directly phosphorylating Ikaros protein and reducing Ikaros’ DNA-binding affinity. The experiments performed in Aim #3, showed that molecular inhibition of CK2, by knocking down CK2 alpha catalytic subunit, results in transcriptional repression of Ikaros target genes CDC2 and CDC7. These results support the hypothesis that in T-ALL, CK2 impairs Ikaros function as a tumor suppressor and regulator of transcription of CDC2 and CDC7 genes.

The experiments performed as proposed in Aims #1-#3, strongly suggest that the tumor suppressor function of Ikaros in T-ALL involves control of the cell cycle progression in leukemia cells. This control is exerted by negative regulation of gene expression of CDC2 and CDC7 genes by transcriptional repression. The results suggest that the oncogenic activity of CK2 in T-ALL, involves inactivation of Ikaros’ ability to repress transcription of CDC2 and CDC7, and thus impaired function of Ikaros as negative regulator of the cell cycle progression in leukemia cells.
Chapter 6: Conclusion

The aim of this study was to provide evidence to support that CK2 plays a key role in the regulation of Ikaros, a master regulator of the cell cycle in T-ALL. This hypothesis was proposed on the basis of previous evidence gathered from studies regarding tumor suppression in B-ALL. Since ALL is a paradigm for the advancement in understanding the genetic basis underlying cancer pathophysiology, this study was geared towards extending the understanding of the different types of leukemia, with a long-term goal to provide a targeted therapy for T-ALL.

The gain-of-function and loss-of-function experiments demonstrated that Ikaros functions as a transcriptional repressor of CDC2 and CDC7 genes in T-ALL. Molecular inhibition of CK2 by knock-down of CK2 alpha subunit, resulted in transcriptional repression of CD2 and CDC7 genes in T-ALL. Results suggest that CK2 promotes leukemogenesis in T-ALL by impairing Ikaros’ ability to regulate cell cycle progression via transcriptional repression of CDC2 and CDC7 genes. The presented data demonstrate the critical roles of Ikaros and CK2 in regulation of tumor suppression and cell cycle progression in T-ALL. Targeting CK2 to restore Ikaros function as a tumor suppressor, can be a potential novel treatment for T-ALL.
References


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Figure 1: Effect of Ikaros Overexpression on CDC2 and CDC7

Ikaros Overexpression downregulates Transcription of CDC2 and CDC7 in T-ALL

![Graph showing the effect of Ikaros overexpression on CDC2 and CDC7 transcription.](image)
Figure 2: Effect of Ikaros Knockdown on CDC2 and CDC7-

**Ikaros Knockdown Increases Transcription of CDC2 and CDC7 in T-ALL**

![Graph showing increase in gene expression of CDC2 and CDC7 after Ikaros knockdown](image-url)
Figure 3: Effect of CK2 alpha knockdown on CDC2 and CDC7-

CK2 alpha Knockdown Decreases Transcription of CDC2 and CDC7 in T-ALL

![Graphs showing the effect of CK2 alpha knockdown on CDC2 and CDC7 expression](image)
<table>
<thead>
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<th>Term</th>
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<tr>
<td>Acetylation</td>
<td>The addition of an acetyl group onto a compound</td>
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<tr>
<td>Acute</td>
<td>Used to describe a disease that progresses rapidly, and lasts for a short duration</td>
</tr>
<tr>
<td>Allele</td>
<td>Alternate form of a gene that is used to code for traits</td>
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<td>Bicistronic retroviral vector</td>
<td>A vector that allows the simultaneous expression of two proteins from the same RNA transcript</td>
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<tr>
<td>CCRF-CEM</td>
<td>A human cell line that from peripheral blood tissue of a female T-ALL patient</td>
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<tr>
<td>Cellular proliferation</td>
<td>A condition between the growth and death (or differentiation) that shows an increase in the number of cells</td>
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<tr>
<td>Chromatin remodeling</td>
<td>The modification of histone machinery that allows condensed DNA to be accessed for the purpose of transcription</td>
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<tr>
<td>Chronic</td>
<td>Used to describe a disease that progresses slowly, and lasts for a long duration.</td>
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<tr>
<td>Cytotoxic</td>
<td>Harmful/lethal to the cell, leading to toxicity that causes damage and in extreme scenarios, cell death</td>
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<td>De novo mutations</td>
<td>New/ novel mutations that are observed for the first time</td>
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<td>Differentiation</td>
<td>The process through which cells in the course of}</td>
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<td>Term</td>
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<tr>
<td>Development change</td>
<td>Change that serves a specific function in the body</td>
</tr>
<tr>
<td>Deacetylation</td>
<td>Removal of an acetyl group from a compound</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>Animal serum used to supplement in vitro culture media</td>
</tr>
<tr>
<td>Haploinsufficiency</td>
<td>Condition in which one of two functional copies of a gene is lost or becomes non-functional</td>
</tr>
<tr>
<td>HDAC</td>
<td>Stands for Histone Deacetylases, a class of compounds that are able to remove an acetyl group that allows the tight binding of DNA</td>
</tr>
<tr>
<td>Heterochromatin</td>
<td>Tightly packed, inaccessible DNA that is considered ‘non-functional’</td>
</tr>
<tr>
<td>Hematopoiesis</td>
<td>Process of formation of blood cells in the body</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>A type of interleukins, which are a special class of proteins that function as immune system signaling molecules</td>
</tr>
<tr>
<td>Kinase</td>
<td>A special type of protein that adds a phosphate group onto a compound, as a form of regulation</td>
</tr>
<tr>
<td>Knockdown</td>
<td>Technique by which the expression of a gene is greatly reduced, to study the effect the absence of the gene in a cell</td>
</tr>
<tr>
<td>Leukemiogenesis</td>
<td>Development of leukemia</td>
</tr>
<tr>
<td>Lymphoblastic</td>
<td>Originating from lymphoid precursors,</td>
</tr>
<tr>
<td>Term</td>
<td>Description</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Increase in the severity and intensity (in the case of cancer, cells that can invade neighboring tissues)</td>
</tr>
<tr>
<td>Oncogenic kinase</td>
<td>A kinase whose activity is associated with cancerous outcomes</td>
</tr>
<tr>
<td>Pathobiology</td>
<td>Associating the pathology of a disease with underlying biological process that is associated with it</td>
</tr>
<tr>
<td>Retroviral production</td>
<td>A technique that allows for the production of retroviruses that can be used for experimentation</td>
</tr>
<tr>
<td>Subunits</td>
<td>The tertiary structure of polypeptides, that can associate with other units to form a multimeric, functional protein</td>
</tr>
<tr>
<td>Suspension cells</td>
<td>Cells that are grown in culture that do not require an adherent surface for their growth and proliferation</td>
</tr>
<tr>
<td>Tetramer</td>
<td>A multimeric protein that is formed as a result of the association of four subunits</td>
</tr>
<tr>
<td>Transcription</td>
<td>The process of using DNA as a template to generate RNA molecules</td>
</tr>
<tr>
<td>Vector</td>
<td>An organism or compound that is used to transmit disease, or disease-causing viruses from a host to another, without causing disease itself</td>
</tr>
<tr>
<td>Zinc finger binding protein</td>
<td>Proteins that have a specific domain that is able to interact with genetic material such as DNA and RNA.</td>
</tr>
</tbody>
</table>
ACADEMIC VITA

SHRIYA KANE  
(717)364-4160 sgk5123@psu.edu  
1001 N Spring Street Middletown, PA

OBJECTIVE
Compassionate, motivated, hard-working, research driven student with a strong desire, commitment and aptitude to pursue a career in medicine with an ultimate goal to aid underserved individuals and minority populations.

EDUCATION
Penn State Harrisburg  
B.S. in Life Science  
August 2014-May 2018

PROFESSIONAL EXPERIENCE AND EXPOSURE

Pediatric Hematology/Oncology- College of Medicine, Hershey  
Honors Thesis-Investigating the role of Casein Kinase II on Ikaros regulation in Acute Lymphoblastic Leukemia  
January 2017-Present

Sidney Kimmel Research Center- Johns Hopkins University  
Summer Internship- Receive training in molecular and cellular techniques and assist in investigating the role of IRAK1 kinase and interleukin-1 (IL-1) receptor signaling pathway in the proliferation and survival of HTLV-1 transformed ATLL cells.  
May 2016-August 2016

Johns Hopkins Hospital- Johns Hopkins University  
Shadowing Experience- Shadowed anesthesia residents under the mentorship of Dr. Christi Grey in the Operating Rooms at JHU Hospital  
May 2016- August 2016

Emergency Medicine Research Associate Program- College of Medicine, Hershey  
Research Assistant- Recruit potential patients from the emergency department for ongoing clinical trials  
January 2016- May 2016

Oyster and Pearl Hospital- Orthopedics Department  
Shadow experience- Shadowed a variety of surgeries performed by Dr. Tapasvi including but not restricted to Sports Medicine and Joint Replacements.  
July 2015-August 2015

Central Pennsylvania Teaching and Research Laboratory for Biofuels  
Research Associate- Proficient in DNA and RNA extractions, PCR, RT-PCR, blotting, media and buffer preparation, biolistics, and plant tissue culture.  
January 2015-December 2015

General Chemistry and Biology Laboratories, Penn State Harrisburg  
Laboratory Facilitator- Assists the learning process in laboratory sessions of general chemistry and molecular biology.  
August 2015- Present

COMMUNITY SERVICE AND EXTRA CURRICULAR ACTIVITIES

STEM Outreach, Penn State Harrisburg:  
Lead outreach events involving under represented high- school students from regional school districts in STEM fields to boost interest in undergraduate careers in science fields.  
February 2015- Present

Mentored 15 high school students from the Susquehanna Township High School in basic techniques of molecular biology and biotechnology  
Mentored 70 7th grade students from the Steelton Highspire Junior High School in basic DNA extraction techniques  
March 2015-April 2015

Honors Service Learning, Peru:  
Part of a project by ACEER and National Geographic that tested the change in the water quality and the nature of eco systems in the Amazon due to the construction of the Trans-Oceanic Highway in Puerto Maldonado.  
Initiated a virtual learning project for high school students in Puerto Maldonado to conduct biology and chemistry based lab sessions  
March 2016-May 2016

Global Lion Mentor Program:  
Serving as a mentor for incoming international students to help them assimilate into the educational and social environment at Penn State Harrisburg  
January 2015-May 2015

Clubs and Sporting Activities:
Serving as Vice President for MAPS - Minority Association of Pre-Medical Students at Penn State Harrisburg
Serving as Fundraising Chair for the Astronomy Club
Member of Intermural teams for Basketball and Badminton

**AWARDS AND RECOGNITION**
Presenter at the Social Impact Lab at the Unite for Sight Global Health and Innovation Conference at Yale University
Received the President Erickson Discovery Grant for Research- Project Title- ‘The effect of Salicylic Acid on Cold and Salt Stressed *Camelina sativa*’
Attended and presented a poster on ‘transcriptomics of the biofuel crop *Camelina sativa* under drought stress” at the Capital College Conference
Dean’s List (all semesters)
Trained in Adult CPR-AED and child CPR by the Red Cross
Recipient of the Hartzler Travel Scholarship for Service Learning
Recipient of the Stephanie A. and Richard E. Ziegler STEM Scholarship