Dr. James Girard Summer Undergraduate Research Program
Faculty Mentor – Project Application

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transfection, electroporation and nucleofection). Additionally, treatment with dimethyl sulfoxide (DMSO) triggers these cells to move through development to complete differentiation into a neutrophil[1] The goal of this research project is to utilize the HL-60 model system to investigate cyclin D3-mediated regulation of gene expression during neutrophil differentiation.

Specific Aim 1: Evaluate how ectopic expression of wild-type or mutant cyclin D3 in uHL-60 cells impacts the expression of genes assessed as progenitor cells (uHL-60) differentiate into neutrophils (dHL-60) in the context of the HL-60 model system.

Specific Aim 2: Determine the ability to abrogate cyclin D3-mediated transcriptional activity by targeting its transcription factor binding partners using pharmacological inhibition.

Specific Aim 3: Assess the ability of cyclin D3 to impact the expression of target genes of interest using a luciferase reporter assay.

elicits a gene expression profile consistent with that triggered when cells are differentiated using DMSO. However, when specific amino acid residues within the cyclin D3 protein were mutated (L215M, Q244R, or P278R), transfection of mutant cyclin D3 into HL-60 cells could no longer regulate the expression of these 14 target genes. Ectopic expression of other cyclin D3 mutants (R72S, E115K, K180R, R252A, and I290R) still demonstrated gene expression profiles consistent with that seen when wild-type cyclin D3 was expressed in HL-60 cells. Altogether, this suggests that cyclin D3 impacts expression of target genes dependent upon the 3D protein structure present when amino acids at positions 215, 244 and 278 are not manipulated, while other protein domains are dispensable.

We have identified additional gene targets to evaluate (see Table 1), and RT-qPCR experiments are underway to assess differences in gene expression as cells differentiate from uHL-60 to dHL-60. It is intended that analysis of genes with differential expression between uHL-60 and dHL-60 states will also be evaluated for gene expression upon ectopic expression of wild-type cyclin D3 during the Spring 2024 semester. For gene targets that seem to be mediated by cyclin D3, experiments will be conducted to determine the impact on target gene expression when cyclin D3 mutants are ectopically expressed (Aim 1).

Table 1. List of Genes Anticipated to Differ in Expression As Progenitor Cells (uHL-60) Differentiate into Neutrophils (dHL-60).

Gene Name	Cellular Function of Resultant Protein	Anticipated Higher Expression
	Enables transcription coregulator activity; regulation of transcription by RNA polymerase II	*uHL-60*
	Interacts with BRCA1 to complete DNA double-strand break repair	*uHL-60*
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Differences observed when ectopic expression uses wild-type or mutant cyclin D3 will help elucidate the mechanism by which cyclin D3 protein can exert control over expression of these target genes.

student will also learn to harvest cells, isolate total RNA, and convert it to cDNA for long term storage/assessment using RT-qPCR. Additionally, genome browser data will be used to identify promoter and enhancer regions that are important for regulating the expression of these genes. This information will be used to sub-clone regulatory regions into luciferase reporter constructs. Furthermore, the SURE student will be exposed to propagating recombinant DNA clones using bacteria, followed by isolation of plasmid DNA to create stocks of wild-type and mutant cyclin D3 clones. Expression vectors (wild-type/mutant clones or luciferase reporters) will then be transfected into cultured cells for RT-qPCR analysis or light emittance, respectively.

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