DNA-Polymerase-α-Primase Complex Subunit Expression and Cytarabine Sensitivity

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Introduction

- Acute myeloid leukemia (AML) involves proliferation of neoplastic myeloid precursor cells that crowd out red blood cells in circulation.[1]
- Even in the same subtype of AML, cell lines and each AML patient respond differently to the frontline treatment cytarabine (ara-C) (OCI-AML2 vs OCI-AML3).[2]
- Cytarabine is a cytidine analog phosphorylated to a triphosphate form and incorporated into the DNA or RNA during DNA synthesis.[3]
- Strand elongation is inhibited and cell death pathways are activated.[3]

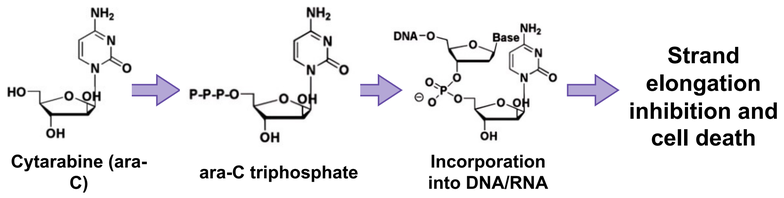
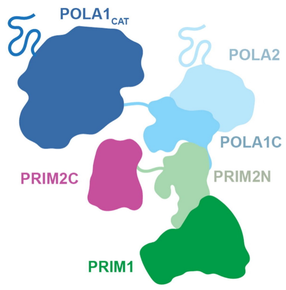


Figure 1: Ara-C phosphorylation to triphosphate form for incorporation into DNA/RNA.

- The DNA-Pol-α-Primase complex initiates DNA replication.[4]
- DNA-Pol-α-Primase complex has primase and polymerase activity.[4]



- Primase activity
  - PRIM1 = catalytic subunit
  - PRIM2 regulatory subunit
- Polymerase activity
  - POLA1 = catalytic subunit
  - POLA2 = regulatory subunit

Figure 2: DNA-Pol-α-Primase complex subunits

Aims

1. Compare **basal subunit expression** of POLA1, POLA2, PRIM1, and PRIM2 between OCI-AML2 and OCI-AML3 cell lines, and **validate dsiRNA knockdown** of each subunit via RT-qPCR.
2. Compare **cell viability** of OCI-AML2 and OCI-AML3 cell lines under ara-C and Ida treatment following knockdown of the DNA-Pol-α-Primase subunits via Resazurin cell viability assay.

Acknowledgements

Thank you to Dr. Maureen McKeague and the McKeague Lab.

Special thanks to Bruktawit Maru, Olivia Kovecses, and Monika Kojic.

Methods

Workflow of Experiments

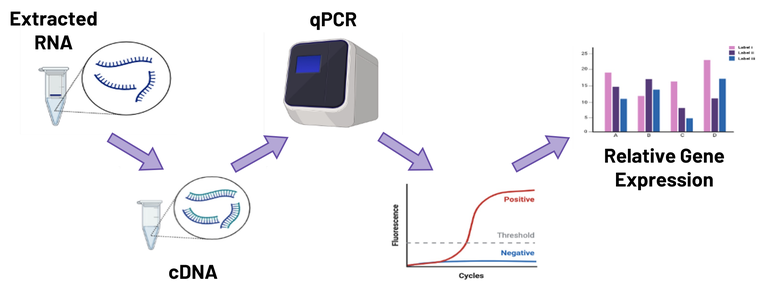


Figure 3: Comparison of PRIM1, PRIM2, POLA1, and POLA2 mRNA expression in OCI-AML2 and OCI-AML3 cell lines via RT-qPCR.

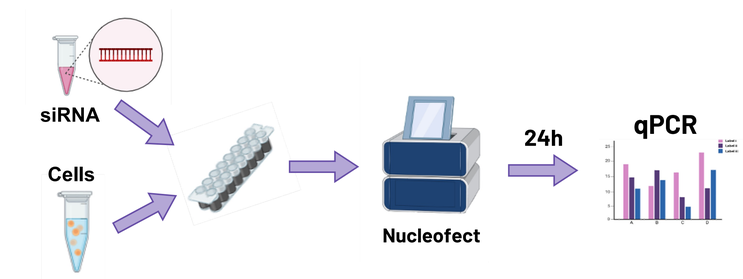


Figure 4: siRNA knockdown of DNA-Pol-α-Primase complex subunits via nucleofection of OCI-AML2 and OCI-AML3 cell lines, and validation via RT-qPCR.

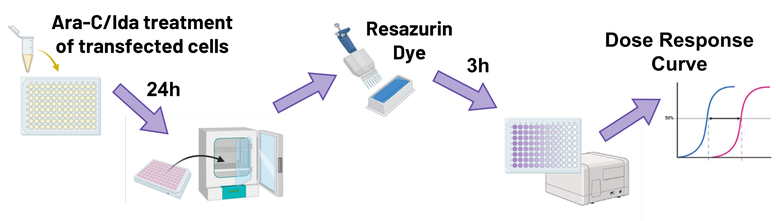


Figure 5: Impact of siRNA knockdown on Ara-C sensitivity determined by treatment of transfected cells with Ara-C and Ida (17:1), followed by a 24 hour incubation and resazurin cell viability assay.

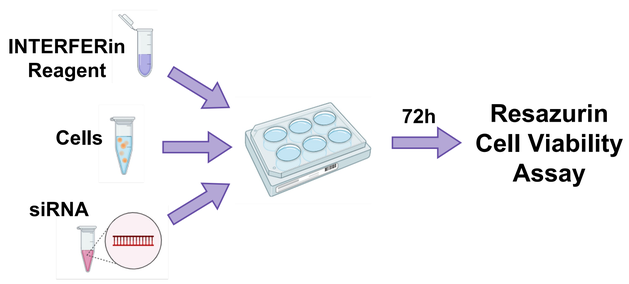
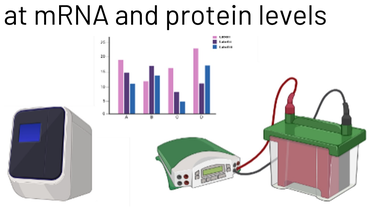


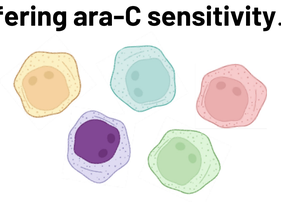
Figure 6: Transfection of OCI-AML3 cells via INTERFERin transfection, then resazurin cell viability assay after a 72 hour incubation.

Future Directions

Validate siRNA knockdown using INTERFERin transfection at mRNA and protein levels



Perform gene knockdown on other AML cell lines with **differing ara-C sensitivity**.



Results and Discussion

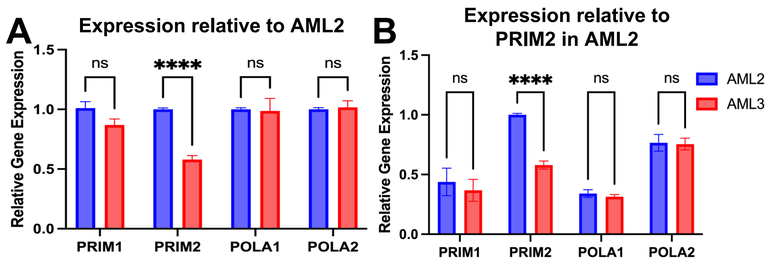


Figure 7: Basal gene expression of DNA-Pol-α-Primase complex subunits. **A)** Basal mRNA expression of DNA-Pol-α-Primase complex subunits in OCI-AML3 relative to the same gene in OCI-AML2 via RT-qPCR. OCI-AML2 showed significantly higher expression of PRIM2. Analysis done via 2 way ANOVA Multiple Comparisons test. ****p<0.0002; n=3, gene expression normalized to GAPDH and HPRT. Error bars shown as SEM. **B)** Same mRNA expression data shown relative to PRIM2 expression in OCI-AML2. The only significant difference between OCI-AML2 and OCI-AML3 is in PRIM2 expression. Analysis done via 2 way ANOVA Multiple Comparisons test. ****p<0.0001; n=3, gene expression normalized to GAPDH and HPRT. Error bars shown as SEM.

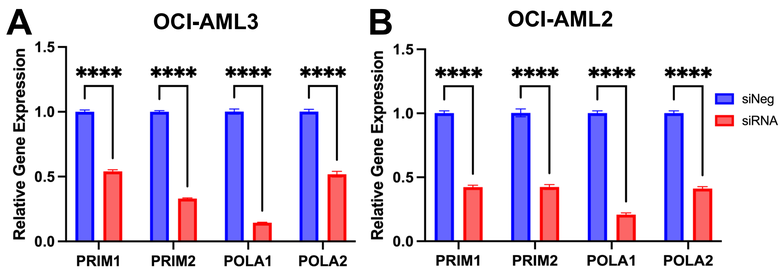


Figure 8: Validation of gene knockdown via siRNA Nucleofection. **A)** Nucleofection of OCI-AML3 cells showed significant knockdown of all DNA-Pol-α-Primase complex subunits at the mRNA level via RT-qPCR. Analysis done via 2 way ANOVA with multiple comparisons. ****p<0.0001. Error bars shown as SEM. **B)** Nucleofection of OCI-AML2 cells showed significant knockdown of all DNA-Pol-α-Primase complex subunits at an mRNA level via RT-qPCR. Analysis done via 2 way ANOVA with multiple comparisons. ****p<0.0001. n=3, gene expression normalized to GAPDH and HPRT. Error bars shown as SEM.

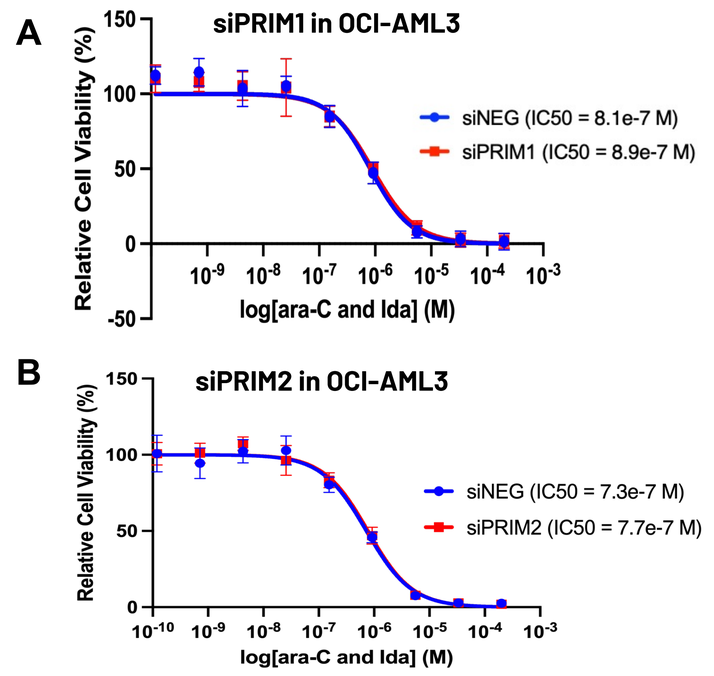


Figure 9: Ara-C sensitivity following siRNA knockdown via INTERFERin Transfection. Cell viability of OCI-AML3 cells was measured via Resazurin cell viability assay following knockdown of **A)** PRIM1 or **B)** PRIM2. Cells were transfected via INTERFERin transfection with siPRIM1 or non-targeting siRNA and incubated for 72h before treatment with ara-C and Ida (17:1), then cell viability was assessed 24h after treatment with ara-C and Ida (17:1). Analysis done via nonlinear regression curve fit for dose response inhibition with variable slope, n=3.

References

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3. Faruqi & Tadi Cytarabine. (2023) *StatPearls [Internet]*.
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Figure 1) Bayles et al. (2022) *Toxicol Appli Pharmacol* 459:116362
Figure 2) Cordoba et al. (2023) *bioRXIV*. Preprint