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Unraveling the Mechanisms of IMP3 Binding Specificity in B-Cell Acute Lymphoblastic Leukemia

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By

Skylar Kensinger

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The Thesis of Skylar Kensinger

is approved:

_____
Professor Jeremy Sanford, chair

_____
Professor Alan Zahler

_____
Professor Dinesh Rao

_____
Peter Biehl
Vice Provost and Dean of Graduate Studies

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Abstract

Unraveling the Mechanisms of IMP3 Binding Specificity in B-Cell Acute Lymphoblastic Leukemia

Skylar Kensinger

Post-transcriptional regulation of gene expression is a complex process involving RNA binding proteins (RBPs) that play a crucial role in mRNA metabolism. RBPs recognize and bind to specific RNA sequences, influencing RNA fate and function. Dysregulation of RBPs has been implicated in various cancers, including hematological malignancies such as B-cell acute lymphoblastic leukemia (B-ALL). The insulin-like growth factor 2 mRNA binding protein 3 (IMP3/IGF2BP3) is an oncofetal RBP that is overexpressed in cancer and has been associated with poor prognosis and resistance to treatment. Despite its significance in cancer, the mechanisms driving IMP3 binding specificity remain poorly understood. In this study, we employed enhanced crosslinking and immunoprecipitation (eCLIP) to investigate the binding specificity of IMP3 in B-ALL cells. By comparing IMP3 targets and binding sites in multiple B-ALL cell lines, we aimed to identify common binding patterns specific to B-ALL and validate genuine leukemogenic IMP3 targets. Our findings shed light on the mechanisms underlying IMP3 binding specificity in B-ALL and provide insights into its role in gene regulation during leukemogenesis. Understanding IMP3-mediated gene regulation in leukemia has the potential to uncover novel therapeutic targets and advance personalized treatment strategies for B-ALL and other cancer types.

Acknowledgments

When I embarked on my journey through graduate school, I was brimming with enthusiasm and a keen desire to nurture my inner scientist. It was a chance for me to delve into uncharted scientific realms, awaiting discoveries yet to be made. Little did I know that this path would unexpectedly transform into a Series of Unfortunate Events. However, thanks to the unwavering support and kindness of everyone involved, I managed to persevere and avoid abandoning my scientific aspirations altogether.

First and foremost, I express my heartfelt gratitude to my mentor, Dr. Jeremy Sanford, whose exceptional patience and unwavering belief in my abilities propelled me forward. I cannot fathom another individual who would tirelessly back my endeavors, especially during times when I proved to be a challenging student. I also extend my deep appreciation to my fellow lab mates, who stood by me when my life seemed to spiral into chaos. In particular, I want to thank Lyna Kabbani for her close collaboration on this project and her invaluable encouragement during my moments of greatest need. Similarly, I am immensely grateful to Zach Neeb, Victor Tse, Eddie Ramirez, and Alex Ritter for their unwavering support and camaraderie throughout my time in the Sanford lab. I could not have asked for a more exceptional group of colleagues.

Furthermore, I extend my heartfelt thanks to my entire committee and the GAC for their flexibility, understanding, and unwavering support. It truly takes a village to achieve such milestones, and it goes without saying that I would not have reached this point alone.

Alastair Reid once said, "Curiosity may have killed the cat… but only the curious have, if they live, a tale worth telling at all." This quote reminds me that despite the challenges faced, my pursuit of knowledge and the support I received have shaped a narrative worth sharing.

1. Introduction

1.1 Post-Transcriptional Regulation

The process of gene expression regulation is governed by the central dogma of molecular biology, which posits that genetic information flows from DNA to RNA to protein. To ensure precise regulation of genetic information, complex molecular machinery and systematic interactions tightly control each phase: transcription of DNA to RNA and translation of RNA to proteins. Historically, the critical role of a third intermediate stage, post-transcriptional regulation (the process between transcription and translation), was underappreciated due to a lack of technology capable of accurately studying gene expression regulation at the RNA level. However, recent technological advancements have expanded our understanding of the importance of post-transcriptional processes underlying gene expression. One such breakthrough is RNA sequencing, which has transformed gene expression analysis by enabling researchers to map and study transcriptomes down to a single cell level [1]. Critically, RNA sequencing technology has revealed a vast network of protein-RNA interactions involved in pre-mRNA processing and has provided insights into how these interactions ultimately impact gene expression [2-6].

1.2 RNA Binding Proteins: Key Regulators of Gene Expression

Among the many molecular interactions that constitute mRNA processing, one of the most notable discoveries was that the establishment of ribonucleoprotein (RNP) complexes formed between RNA binding proteins (RBPs) and RNA significantly impacts RNA fate and function. Consequently, RBPs are considered key regulators of post-transcriptional regulation and play an essential role in gene expression. RBPs are proteins that recognize and bind to various sequence and structural motifs on RNA via RNA binding domains (RBDs) such as RNA recognition motifs (RRMs), hnRNP K homology domains (KHs), DEAD box helicase domains, and many others that have been (and yet to be) discovered [7,8]. RBPs play a role in each stage of RNA metabolism, including maturation, transport, stability, and degradation of RNA

molecules [9,10]. Furthermore, many RBPs are multifunctional and can regulate different steps in gene expression [11]. The effect that an RBP has on its target RNA depends on a wide variety of factors, many of which have yet to be uncovered. Studies have shown that RBPs preferentially bind to specific regions of RNA transcripts, suggesting that binding location plays a vital role in determining the effect on RNA fate and function [12]. RNA binding specificity depends, in part, on the specific RBDs within each protein [13, 14]. For example, the KH domains of the IMP3 protein have been associated with binding to the DRACH motif on specific RNA transcripts, while the RRMs are hypothesized to play a role in stabilizing binding to the specific motif [15-18].

KH domains and RNA recognition motifs (RRMs) are critical components of RBPs that are crucial for determining binding specificity. KH domains are structurally conserved domains found in various RBPs, enabling them to recognize and bind to specific RNA sequences. These domains form an RNA-binding pocket that interacts with the target RNA, facilitating stable and specific interactions. Similarly, RRMs are another class of RNA binding domains commonly found in RBPs. RRMs exhibit a modular structure and can recognize specific RNA motifs through interactions with RNA bases and backbone. The combination of KH domains and RRMs allows RBPs to recognize and bind to distinct RNA sequences, contributing to their binding specificity. The precise arrangement and composition of these domains within RBPs contribute to the diversity of RNA targets that can be recognized and regulated. The intricate interplay between KH domains, RRMs, and other RNA binding domains empowers RBPs to selectively interact with their target RNAs, ultimately influencing gene expression and RNA fate. However, further study is required to understand the complete mechanism that drives RBP binding specificity; and doing so will aid in elucidating the roles and mechanisms of multifunctional RBPs in gene expression regulation.

1.3 Hematological Cancers

RBP dysregulation has been observed among numerous cancer types, and aberrant expression of RBPs in these cancers is often associated with poor prognosis and resistance to treatment [19-21]. A fascinating example is the dysregulation of RBPs in the development and progression of hematological cancers such as leukemia [23,24]. However, the roles and mechanisms of many RBPs in tumor progression are challenging to understand due to their versatility and tissue-specific functions [22]. Elucidating the mechanisms behind the roles of RBPs in cancer will allow for their use as therapeutic targets in developing effective treatment strategies and combating treatment resistance.

Recent advances in the methodology used to study leukemic cell lines have allowed for the classification of leukemogenic types based on disease progression, cell type, and the genomic patterns exhibited by each. The four main types of leukemia include chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic lymphoblastic leukemia (CLL), and acute lymphoblastic leukemia (ALL) [25]. Myeloid leukemia occurs among the myeloid cells, which mature in the bone marrow and give rise to certain types of white blood cells. Lymphoblastic leukemia (aka lymphocytic) occurs among lymphocytes, a primary type of white blood cell that matures outside the bone marrow and gives rise to all immunological white blood cells. Chronic leukemia cases are characterized by slow progression and do not require immediate treatment. In contrast, acute leukemia cases progress faster and more aggressively than their chronic counterparts and typically require immediate treatment strategies. Each type of leukemia can be further classified into specific subtypes based on its origin. For instance, mixed lineage leukemia (MLL) involves a chromosomal rearrangement at the 11q23 locus, which results in the formation of oncogenic fusions between the MLL gene and genes that are not canonically related [26]. Consequently, a series of leukemogenic signaling pathways specific to each fusion pair are triggered and typically involve the dysregulation of post-transcriptional processes mediated by aberrant RBPs [27]. Such is the case with AF4-MLL B-cell acute lymphoblastic leukemia (AF4-MLL B-ALL), where MLL fuses

with the AF4 gene, a transcription factor involved in leukemogenesis, activating the IMP3 protein. [28].

1.4 IMP3, a Multidomain Oncofetal RBP

The insulin-like growth factor 2 mRNA binding protein 3 (aka IMP3/IGF2BP3) is a multifunctional RBP initially identified in 1997 by Mueller-Pillasch et al. while screening for genes differentially expressed in pancreatic cancer [30]. Since then, IMP3 has been defined as an oncofetal RBP that maintains mRNA stability during embryogenesis and is overexpressed among multiple cancer types [31-33]. Previous studies have demonstrated that IMP3 plays a crucial role in regulating oncogenic gene expression, and its aberrant overexpression is associated with poor prognosis and resistance to treatment, particularly in mixed-lineage leukemia (MLL) [34]. Because IMP3 has a clear role in cancer, this RBP has promising potential as a therapeutic target. However, a limited understanding of IMP3 mechanisms and target specificity hinders the road to clinical translation.

IMP3's role and mechanism in oncogenic gene regulation depend on the context of its binding partners and disease type [35]. It is hypothesized that IMP3 can regulate oncogenic gene expression through mRNA localization, degradation, stability, and alternative splicing, which are all contingent upon its interactions with the target transcript [36]. However, what drives IMP3 binding specificity remains an important question. Evidence suggests that the four KH domains on the IMP3 protein are responsible for recognizing and binding to specific binding sites on target mRNA transcripts and that its two RRMs help guide the protein to these sites and stabilize the interaction [18,38]. Furthermore, IMP3 binding specificity seems to occur preferentially, specifically favoring the DRACH motif and the 3' UTR of target transcripts [34,37,38,39]. Each binding domain may be able to recognize, guide, and bind to distinct sequence motifs depending on the genetic environment. Therefore, understanding the role of individual binding domains under specific conditions is fundamental to elucidating the mechanisms that drive RBP binding specificity.

1.5 Mechanisms of IMP3 Activity in Oncogenesis

IMP3 has emerged as a significant player in many types of cancers, including pancreatic cancer and leukemia [30, 34]. Studies have revealed that IMP3 plays a role in various aspects of cancer progression, including tumor growth, invasion, and metastasis. In pancreatic cancer, IMP3 has been implicated in promoting epithelial-mesenchymal transition (EMT), a process that enhances cancer cell migration and invasion. IMP3 has also been found to regulate key signaling pathways involved in pancreatic cancer, such as the PI3K/AKT and Wnt/β-catenin pathways, further contributing to tumor development and progression [46, 47]. Additionally, IMP3 has been explored as a potential biomarker for pancreatic cancer diagnosis and a therapeutic target for novel treatment strategies [48]. Likewise, IMP3 has been implicated in the context of leukemia, particularly B-cell acute lymphoblastic leukemia (B-ALL) [34]. In B-ALL, IMP3 dysregulation has been observed, with increased expression levels detected in leukemic cells compared to normal cells. Studies have suggested that IMP3 plays a role in the pathogenesis and progression of B-ALL. It has been associated with abnormal cell proliferation, enhanced cell survival, and resistance to chemotherapy [23]. Moreover, IMP3 has been implicated in regulating critical signaling pathways involved in leukemogenesis, such as the Notch pathway. Studies by our lab in collaboration with the Rao lab at UCLA have demonstrated through multiple binding assays that IMP3's binding pattern in B-ALL reflects a preference for the 3'UTR of its target mRNAs [29]. Furthermore, IMP3 binding to the 3'UTR often overlaps with binding sites for micro RNAs (miRNAs) that target the same transcript. This observation led to the hypothesis that IMP3 may control the RNA-induced silencing complex (RISC) access to mRNA, amplifying oncogenic gene expression. The dysregulation of IMP3 in B-ALL underscores its potential as a therapeutic target and a prognostic marker. Further investigations into the precise mechanisms by which IMP3 influences B-ALL development and its interaction with other

molecular players will enhance our understanding of leukemogenesis and potentially lead to the development of novel treatment approaches for this aggressive form of leukemia.

1.6 Methods for Analyzing Protein-RNA Interactions

The most commonly used strategies to study protein-RNA interactions are immunoprecipitation-based assays such as RNA immunoprecipitation (RIP) assays or crosslinking and immunoprecipitation (CLIP) assays [40]. Both approaches have improved over the years as technology advanced, including the addition of high-throughput sequencing (RIP-seq/CLIP-seq). While both approaches can isolate and identify protein-bound RNA sequences, CLIP uses ultraviolet light to irreversibly covalently link RBPs to target RNAs, stabilizing the bound complex before isolation by RNase treatment and immunoprecipitation. Over time, CLIP has evolved to fit various applications. However, the two main CLIP methods used to identify RBP binding sites are iCLIP (individual nucleotide resolution crosslinking and immunoprecipitation) and eCLIP (enhanced crosslinking immunoprecipitation). While both iCLIP and eCLIP can map protein-RNA interactions at a single nucleotide resolution, eCLIP offers increased specificity by utilizing two single-strand adapter sequences before PCR amplification and a size-matched input control, reducing duplication rates, amplification bias, and non-specific background signal that is commonly observed with iCLIP [42].

In this study, we utilized eCLIP to investigate the binding specificity of IMP3 in B-cell acute lymphoblastic leukemia (B-ALL) cells. To gain a comprehensive understanding, we compared IMP3 targets and binding sites identified in SEM cells with the binding patterns observed in a previous study conducted by our lab using REH and RS4;11 cells [34]. By examining IMP3 targets and binding sites across multiple B-ALL cell lines, we aimed to validate genuine leukemogenic IMP3 targets and identify common IMP3 binding patterns specific to B-ALL. This comparative approach sheds light on shared IMP3 binding patterns unique to B-ALL and reveals potential targets and binding patterns specific to particular leukemogenic cell lines. This information is crucial for developing innovative therapeutic strategies tailored to each

subtype of B-ALL, thereby promoting a more personalized approach to leukemia treatment and potentially extending to other cancer types.

By unraveling the similarities and differences in IMP3 binding patterns among various B-ALL cell lines, we gain deeper insights into the underlying mechanisms of IMP3 binding specificity in B-ALL and its role in regulating gene expression during leukemogenic progression. This knowledge not only advances our understanding of IMP3's function in B-ALL but also paves the way for leveraging IMP3 and other RBPs as therapeutic targets for the development of novel treatment modalities in leukemia and other cancer types. Thus, unraveling the mechanism behind IMP3-mediated gene regulation in leukemia holds great promise for accelerating therapeutic advancements in the field.

2. Materials and Methods

2.1 Cell Preparation

SEM I3KO/NT suspension cells were cultured in 90% IMDM supplemented with Penicillin, Streptomycin, and 10% FBS. A total of 7x10^7 cells were titrated to yield 100 ug RNA per condition (NT and I3KO). Three replicates per condition were crosslinked via ultraviolet light at 235 -nm wavelength and sent out to ECLIPSE Bio for eCLIP analysis.

2.2 Enhanced crosslinking-immunoprecipitation (eCLIP)

eCLIP-seq was performed according to the protocol by Eclipse BioInnovations on three technical replicates and a size-matched input (smInput) control using Anti-IMP3 (Human/Mouse) pAb (Polyclonal Antibody) [43]. Peak data was analyzed via Eclipse BioInnovation’s software pipeline.

3. Results

3.1 Enhanced Crosslinking and Immunoprecipitation of IMP3

We employed eCLIP-seq to uncover the specific mRNA targets associated with IMP3 in SEM cells (Figure 1A). The presence of IMP3 protein was confirmed through Western blot analysis, which demonstrated its presence in 10% of the IMP3 immunoprecipitated (IP) samples from SEM cells. As a control, 1% of input samples from HEPG2 cells were used (Figure 1B). To assess the presence of RNA bound to the protein, nucleic acid staining of the bound proteins was performed and quantified using Tapestation (Figure 1C, 1D). The successful confirmation of IMP3 protein presence in the immunoprecipitated samples from SEM cells, along with the control HEPG2 cells, validates the specificity of our experimental approach. The nucleic acid staining further supports the binding of RNA to the IMP3 protein, providing additional evidence for the RNA-binding capacity of IMP3 in SEM cells. Overall, these experimental findings establish the foundation for the subsequent analysis of IMP3-bound mRNA targets in SEM cells, providing confidence in the specificity and reliability of our eCLIP-seq approach.

3.2 IMP3 Binds to Hundreds of mRNA Transcripts in SEM Cells.

In our analysis, a total of 772 direct targets of IMP3 were identified, out of which 640 targets were not observed in RS4;11, and REH cells (Figure 2). The distribution of IMP3 binding sites was further investigated, revealing that the majority of these sites were located in the 3' untranslated region (3' UTR), comprising 52.4% of the sites, followed by the coding sequence (CDS) region accounting for 45.6% of the sites. In contrast, only a small fraction of the peaks (0.231%) were found in the 5' untranslated region (5' UTR) (Figure 3A). Metagene analysis of the enhanced crosslinking and immunoprecipitation (eCLIP) data provided additional insights into the distribution of the most enriched sequence elements. Notably, these elements were predominantly found within the 3' UTR of the target genes (Figure 3B). The preference for IMP3 binding within the 3' UTR is consistent with our previous findings in RS4;11 and REH

cells, suggesting a conserved pattern of IMP3 binding in different cellular contexts. These results highlight the localization of IMP3 binding sites within the 3' UTR and CDS regions of target transcripts, emphasizing the potential regulatory role of IMP3 in post-transcriptional processes. The enrichment of sequence elements within the 3' UTR further supports the functional significance of IMP3-mediated regulation in this specific region. The consistent 3' UTR binding pattern by IMP3 across different cell types strengthens the evidence for its regulatory involvement in gene expression.

3.3 IMP3 Binding Site Preferences

To gain insights into the potential influence of IMP3 binding on gene regulation, we employed HOMER analysis to identify the specific binding site sequences that were predominantly targeted by IMP3. Previous studies have highlighted the tendency of IMP3 to bind to CA-rich sequences, and our analysis corroborated these observations [38]. Our analysis determined that the most enriched sequence motif targeted by IMP3 is "CUUCUGUA" (Figure 4). This motif was found to be present in the genomic regions of approximately 35.81% of the target genes identified during eCLIP analysis. The identification of the "CUUCUGUA" motif as the primary binding site for IMP3 suggests its significance in the regulatory functions mediated by this RNA-binding protein. Additionally, our analysis confirmed the previously reported tendency of IMP3 to bind to CA-rich sequences. This finding further strengthens the existing knowledge regarding the binding preferences of IMP3 and reinforces the consistency of our HOMER analysis results with previous studies. The identification of the "CUUCUGUA" motif as the most enriched target for IMP3 binding and the association of IMP3 with CA-rich sequences underscore the potential role of IMP3 in the regulation of gene expression. Understanding the functional implications of IMP3 binding to the "CUUCUGUA" motif and CA-rich sequences will provide valuable insights into the mechanisms by which this RNA-binding protein contributes to gene regulatory networks.

3.4 Characterization of IMP3's Role in Leukemogenic Gene Expression

To gain a deeper understanding of IMP3's involvement in the regulation of leukemogenic gene expression, we conducted a gene ontology (GO) analysis on IMP3 target transcripts identified through eCLIP. The analysis aimed to uncover the functional categories and pathways associated with these IMP3 target transcripts. Our GO analysis results highlighted two predominant functional categories among the IMP3 target transcripts. Specifically, we observed that a significant proportion of the target transcripts, 24 out of 346 genes, were involved in protein processing (Figure 5). This finding suggests that IMP3 may play a crucial role in the regulation of protein processing pathways within the context of leukemogenesis. Additionally, we identified 23 out of 346 genes that were associated with transcriptional misregulation in cancer. This observation further implicates IMP3 in the dysregulation of transcriptional processes linked to leukemogenesis. These findings shed light on the potential molecular mechanisms through which IMP3 contributes to the regulation of leukemogenic gene expression. The identification of protein processing and transcriptional misregulation in cancer as enriched functional categories among IMP3 target transcripts suggests that IMP3 may exert its influence on leukemogenic pathways by modulating these specific processes.

3.5 Specific IGF2BP3 mRNA Targets of Interest in SEM Cells

Previous studies have provided evidence that MYC and CDK6 are direct targets of IMP3 in B-cell acute lymphoblastic leukemia (B-ALL) cell lines. To validate these findings in the context of our study using SEM cells, we performed eCLIP analysis, which demonstrated significant enrichment of IMP3 binding on MYC and CDK6 transcripts (Figures 6 & 7). This reinforces their status as direct targets of IMP3 in B-ALL. Intriguingly, our analysis also revealed PAICS as a potential target, which exhibited the most notable enrichment for IMP3 binding, as indicated by a p-value of 400. The peaks of IMP3 binding on PAICS were exclusively observed in various regions of the 3' untranslated region (UTR) (Figure 8). This

specific binding pattern suggests a potential regulatory role of IMP3 in the post-transcriptional regulation of PAICS in B-ALL. Moreover, our previous microarray data provided additional support for the significance of PAICS in B-ALL. These data demonstrated that PAICS is highly expressed in B-ALL, ranking just 13 genes below IMP3 in terms of expression levels. This correlation between the expression levels of PAICS and IMP3 suggests a potential functional relationship, highlighting the possibility of IMP3-mediated regulation of PAICS expression in B-ALL. Collectively, our findings confirm MYC and CDK6 as direct targets of IMP3 in B-ALL, in line with previous studies. Additionally, our eCLIP analysis identifies PAICS as a novel target with significant IMP3 binding enrichment, specifically within the 3' UTR. The high expression of PAICS in B-ALL, coupled with its proximity to IMP3 expression levels, further supports the potential involvement of IMP3 in the regulation of PAICS expression in this leukemia subtype. These discoveries contribute to our understanding of the regulatory network governed by IMP3 and its implications in B-ALL pathogenesis.

4. Discussion

4.1 Implications of IMP3 Binding Specificity in B-ALL

The identification of 772 direct IMP3 targets in SEM cells, with a significant number of targets not observed in previous studies using RS4;11 and REH cells, highlights the context-dependent nature of IMP3 binding specificity in B-ALL. These findings suggest that IMP3's role in oncogenic gene regulation may vary across different B-ALL subtypes. The enrichment of IMP3 binding on well-established targets such as MYC and CDK6 further validates their status as direct IMP3 targets in B-ALL, reinforcing the importance of IMP3 in B-ALL pathogenesis.

Among the identified targets, PAICS stands out as the gene showing the most significant enrichment for IMP3 binding. The exclusive binding of IMP3 on various regions of the PAICS 3' UTR suggests a potential role for IMP3 in regulating PAICS expression in B-ALL. PAICS is known to be highly expressed in B-ALL, and its overexpression in other cancer types, such as

gastric cancer, has been associated with aggressive disease and poor prognosis [44]. Furthermore, previous studies have demonstrated that PAICS is a direct target of MYC, which binds to the PAICS promoter, and interference with MYC's binding to the PAICS promoter in gastric cancer cells decreases oncogenic progression and function [45]. It is possible that IMP3 competes for binding to PAICS in B-ALL and interferes with canonical binding factors, thus altering PAICS function. Further investigations are required to determine the functional consequences of the interplay between IMP3 and PAICS and may provide valuable insights into the pathogenesis of B-ALL.

4.2 Binding Preference of IMP3 in B-ALL

Analysis of the peak data from eCLIP-seq revealed a clear preference for IMP3 binding to target transcripts' 3' UTR and CDS regions, consistent with previous findings in RS4;11 and REH cells. This preference for 3' UTR binding suggests that IMP3 may predominantly exert its regulatory effects on mRNA stability, localization, and/or translation efficiency in B-ALL. The enrichment of IMP3 binding in the CDS region further suggests a potential role of IMP3 in modulating alternative splicing and mRNA processing. These findings emphasize the multifaceted role of IMP3 in post-transcriptional gene regulation and highlight the need for further investigations to dissect the specific mechanisms by which IMP3 influences mRNA fate and function in B-ALL.

4.3 Therapeutic Targeting of IMP3 in B-ALL

Understanding the mechanisms of IMP3 binding specificity in B-ALL is crucial for developing targeted therapies aimed at disrupting the dysregulated gene expression patterns mediated by IMP3. Identifying direct IMP3 targets, such as MYC, CDK6, and PAICS, provides potential avenues for therapeutic intervention. Targeting IMP3 or its downstream effectors could potentially disrupt the oncogenic pathways activated in B-ALL, leading to improved treatment outcomes and overcoming treatment resistance.

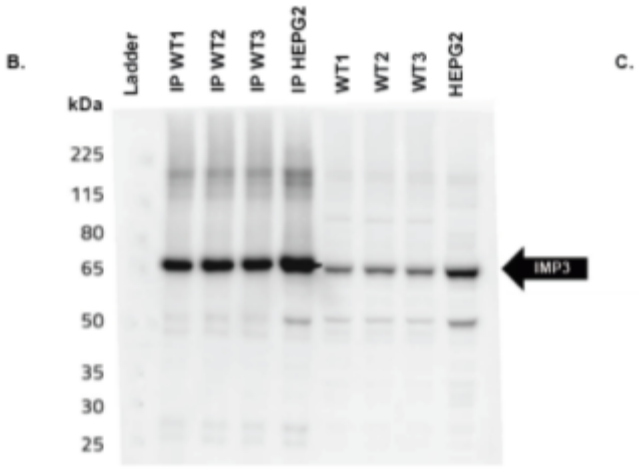
Moreover, the comparative analysis of IMP3 targets and binding sites across multiple B-ALL cell lines, as demonstrated in this study, offers insights into the shared and unique binding patterns specific to B-ALL subtypes. This information contributes to our understanding of B-ALL heterogeneity and opens up possibilities for developing personalized treatment strategies. Tailoring therapeutic approaches based on the distinct IMP3 binding patterns observed in different B-ALL subtypes could maximize treatment efficacy and minimize adverse effects, ultimately improving patient outcomes.

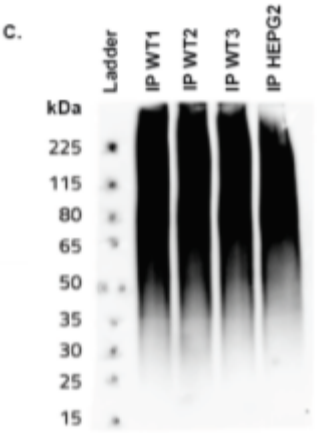
4.4 Future Directions

The diverse set of IMP3 target genes, including critical oncogenes such as MYC and CDK6 and metabolic genes like PAICS, suggests a multifaceted role for IMP3 in driving B-ALL pathogenesis. While this study provides valuable insights into the mechanisms of IMP3 binding specificity in B-ALL, several questions and avenues for future research remain. First, investigating the functional consequences of IMP3 binding to specific target transcripts, particularly in relation to mRNA stability, localization, and translation efficiency, will enhance our understanding of the impact of IMP3-mediated gene regulation in B-ALL. Further studies exploring the downstream effects of disrupting IMP3 binding or expression on B-ALL cell growth, survival, and differentiation are also warranted and will provide a deeper understanding of the functional consequences of aberrant IMP3-mediated gene regulation on downstream processes.

A.

Library	Initial	Pass Trim	Rep Elements	Unique Aligned	PCR Duplicates	Final Reads	Total Clusters	Total Peaks
HEPG2_S29_R1_001	32.23M	99.84%	49.69%	81.94%	19.98%	10.62M	211,563	9,467
WT1_S26_R1_001	42.28M	99.70%	50.60%	76.62%	19.82%	12.79M	240,030	6,191
WT2_S27_R1_001	42.45M	99.75%	54.38%	76.52%	20.09%	11.81M	222,864	5,611
WT3_S28_R1_001	5.86M	99.74%	50.85%	77.84%	18.76%	1.82M	70,578	5,564
INPUT_HEPG2_S36_R1_001	36.54M	99.91%	54.40%	87.72%	17.71%	12.02M	NA	NA
INPUT_WT1_S33_R1_001	39.77M	99.88%	72.67%	76.37%	19.63%	6.66M	NA	NA
INPUT_WT2_S34_R1_001	35.05M	99.88%	72.28%	77.25%	18.96%	6.07M	NA	NA
INPUT_WT3_S35_R1_001	37.89M	99.84%	69.37%	79.53%	20.56%	7.32M	NA	NA





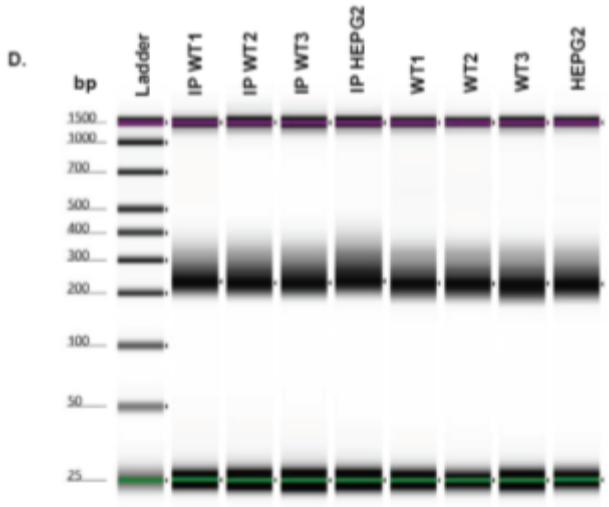


Figure 1: Immunoprecipitation and Quantification during IMP3 eCLIP analysis.
A. Table summarizing the quantitative results from eCLIP analysis for each sample. **B.** Western blot confirming the presence of the IMP3 protein in 10% of IP samples and 1% of input samples using Anti-IMP3 pAb and an anti-rabbit IgG (HRP) antibody with HEPG2 as controls. **C.** Nucleic acid stain from western blot analysis visualizing the amount of RNA in each IP sample. **D.** Tapestation quantitation of libraries created for each IP sample, input sample, and HEPG2 controls during eCLIP.

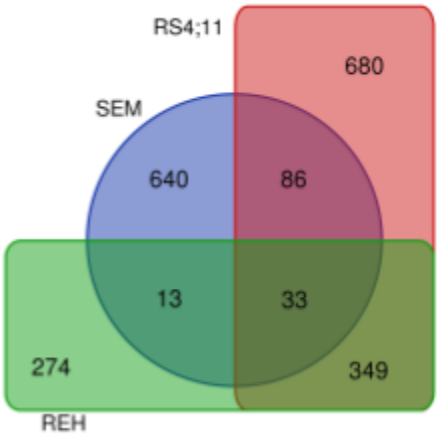


Figure 2: IMP3 Target Ratios between SEM, RS4;11, and REH cells. A venn diagram portraying the number of unique and shared IMP3 target transcripts between the SEM cells used in this study, RS4;11 cells from a previous study, and REH cells.

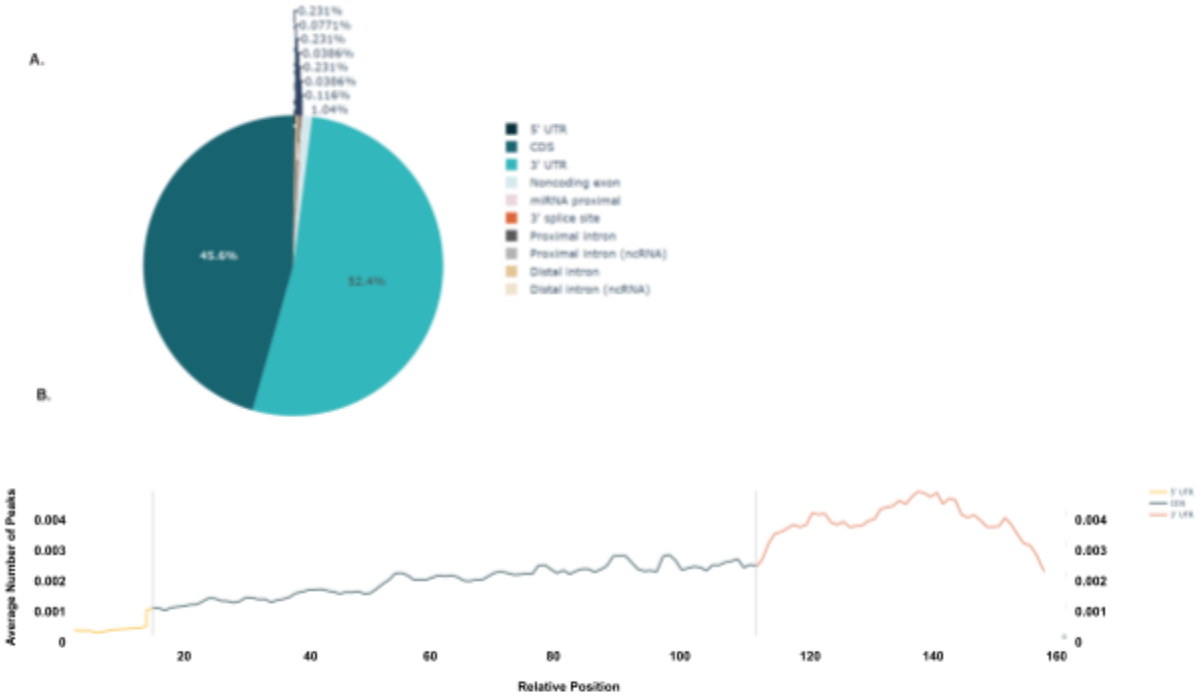


Figure 3: Peak distribution from eCLIP analysis. A. Pie chart displaying the relative peak frequency mapped to each sequence feature amongst IP samples (p-value ≤ 0.001). **B.** Metagene plot reflecting the average number of peaks mapped to specific genomic regions amongst every target gene.

Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)
	1e-22	-5.109e+01	35.81%	26.95%	34.2bp (21.8bp)
	1e-25	-5.948e+01	21.49%	13.76%	33.8bp (23.8bp)
	1e-14	-3.335e+01	13.35%	8.68%	30.1bp (22.0bp)
	1e-9	-2.223e+01	12.33%	8.65%	34.2bp (21.6bp)
	1e-14	-3.238e+01	11.39%	7.16%	35.1bp (21.8bp)
	1e-16	-3.840e+01	10.45%	6.08%	34.1bp (23.8bp)
	1e-30	-6.947e+01	8.30%	3.42%	31.4bp (22.0bp)
	1e-13	-3.032e+01	6.69%	3.63%	25.7bp (21.0bp)

Figure 4: Motif Enrichment from eCLIP analysis. HOMER analysis displaying most enriched motifs amongst all three replicates.

Search:

Description	Gene ratio	Bg ratio	P-value	Adjusted P-value	Q-value	Count
Protein processing in endoplasmic reticulum	24/346	171/8386	1.21e-7	3.45e-5	2.91e-5	24
Transcriptional misregulation in cancer	23/346	193/8386	4.12e-6	5.88e-4	4.95e-4	23
Pathogenic Escherichia coli infection	22/346	197/8386	1.91e-5	0.002	0.002	22
Salmonella infection	25/346	249/8386	3.27e-5	0.002	0.002	25
Hepatitis C	18/346	157/8386	7.77e-5	0.004	0.004	18
Adherens junction	13/346	93/8386	1.05e-4	0.005	0.004	13
Epstein-Barr virus infection	20/346	202/8386	2.43e-4	0.010	0.008	20
AMPK signaling pathway	14/346	121/8386	4.38e-4	0.013	0.011	14
Regulation of actin cytoskeleton	21/346	229/8386	4.88e-4	0.013	0.011	21
Hepatocellular carcinoma	17/346	168/8386	5.48e-4	0.013	0.011	17

Figure 5: Enriched sequence elements from eCLIP analysis. KEGG enrichment analysis displaying the most enriched function pathways amongst all three IP replicates.

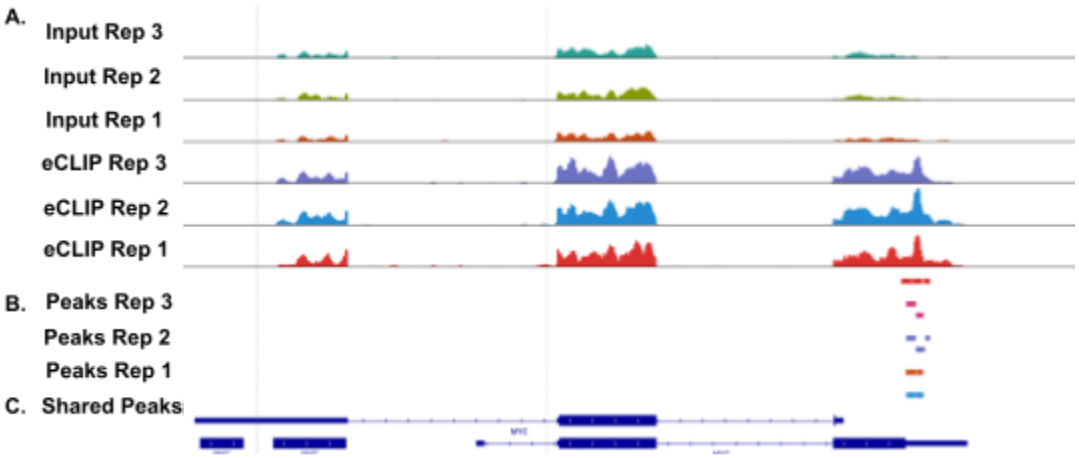


Figure 6: Peak distribution for MYC from eCLIP analysis. A. Peak enrichment in abundant regions amongst each IP replicate, excluding input samples. **B.** Reproducible peaks across all three IP replicates. **C.** Gene annotation representing possible isoforms for MYC.

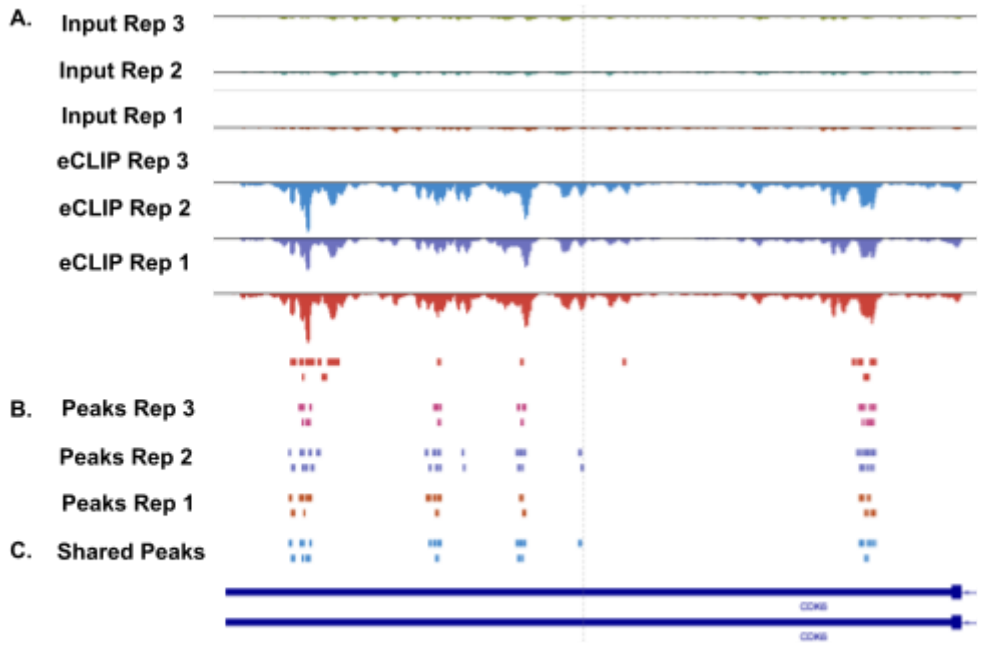


Figure 7: Peak distribution for CDK6 from eCLIP analysis. A. Peak enrichment in abundant regions amongst each IP replicate, excluding input samples. **B.** Reproducible peaks across all three IP replicates. **C.** Gene annotation representing possible isoforms for CDK6.

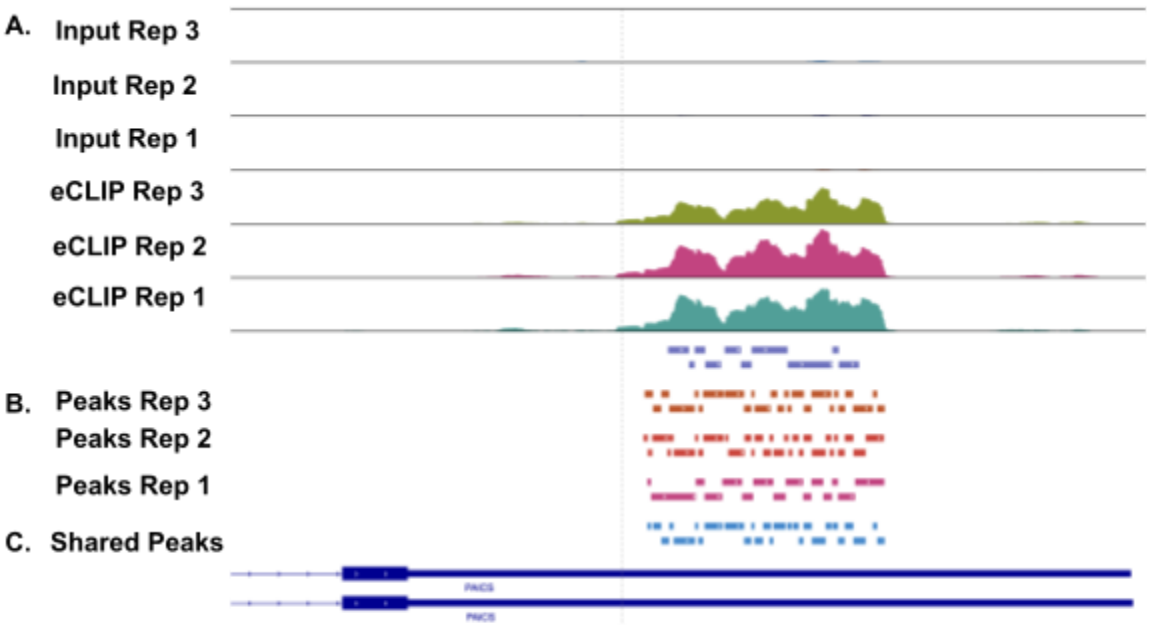


Figure 8: Peak distribution for PAICS from eCLIP analysis. A. Peak enrichment in abundant regions amongst each IP replicate, excluding input samples. **B.** Reproducible peaks across all three IP replicates. **C.** Gene annotation representing possible isoforms for PAICS.

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